PRIMARY CUTANEOUS AND ANAL MALIGNANT MELANOMA: A COMPLETE HISTOMORPHOLOGICAL STUDY WITH CLINICOPATHOLOGICAL CORRELATION AND BRAF MUTATION ANALYSIS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REGULATION FOR THE AWARD OF THE DEGREE OF MD PATHOLOGY (BRANCH III)

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI, TAMILNADU

MAY – 2018

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This is to certify that the dissertation titled "**Primary cutaneous and anal malignant melanoma: A complete histomorphological study with clinicopathological correlation and BRAF mutation analysis**" is the bonafide work of Dr. Nufina T A, submitted in partial fulfillment of the rules and regulations for the M.D Branch III (Pathology) Degree examination of The Tamil Nadu Dr. MGR Medical University, to be held in May 2018.

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This is to declare that this dissertation titled "**Primary cutaneous and anal malignant melanoma: A complete histomorphological study with clinicopathological correlation and BRAF mutation analysis**" is my original work done under the guidance of Dr Meera Thomas, in partial fulfillment of the rules and regulations for the M.D Branch III (Pathology) Degree examination of the Tamil Nadu Dr. MGR Medical University, to be held in May 2018. I have independently reviewed the literature, standardized the data collection methodology and carried out the evaluation towards completion of the thesis.

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A sum of 80,750/- INR (Rupees Eighty Thousand Seven hundred and fifty Only) will be granted for 2 years.

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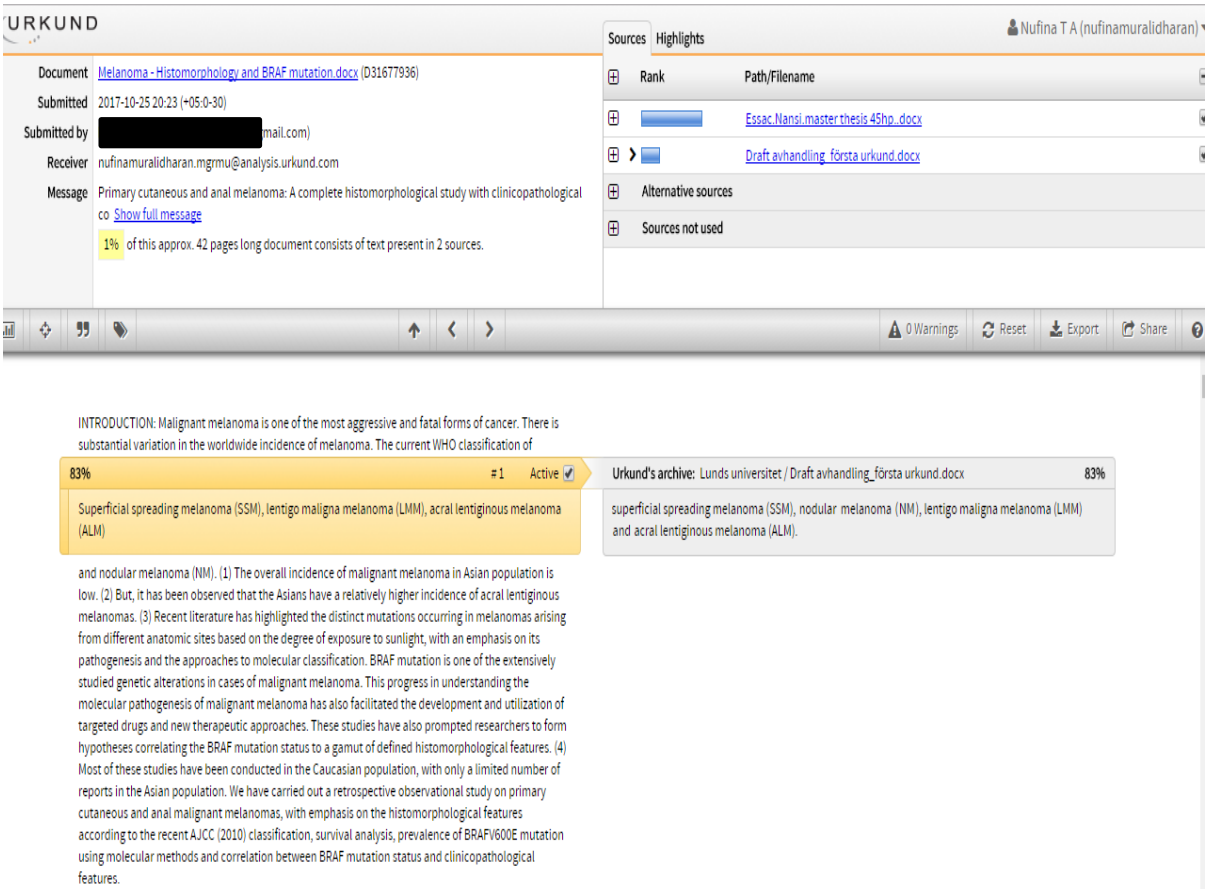

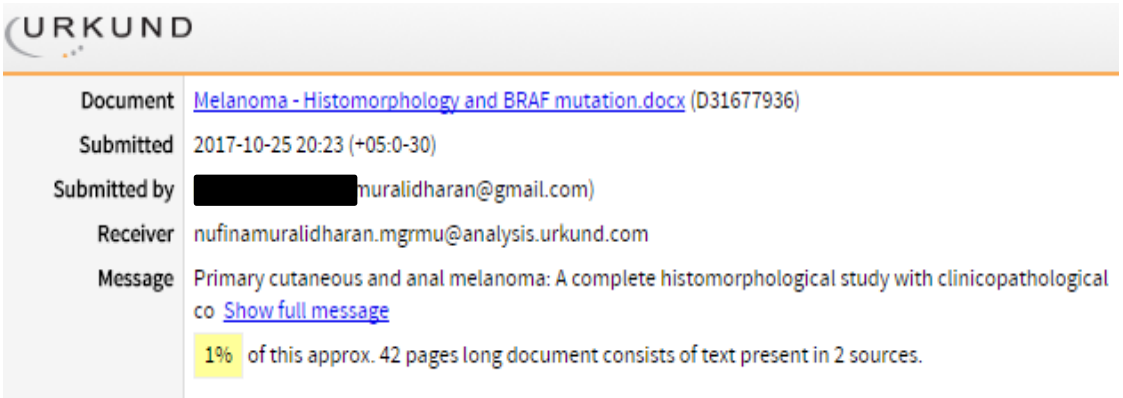

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This is to certify that this dissertation titled "**Primary cutaneous and anal malignant melanoma: A complete histomorphological study with clinicopathological correlation and BRAF mutation analysis**" submitted by Dr. Nufina T A (Registration No. 201513354.) for the award of Degree of M.D Branch III (Pathology). I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains the pages from introduction to conclusion and the result shows **ONE** percent of plagiarism in the dissertation.

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ABSTRACT

Primary cutaneous and anal malignant melanoma - A complete histomorphological study and BRAF mutation analysis

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OBJECTIVES: To carry out a retrospective complete histomorphological study with survival analyses and BRAF mutation status of primary cutaneous and anal melanoma.

METHODS: A cohort study was conducted on primary cutaneous and anal melanomas (January 2013 to December 2015) wherein a complete histomorphological study, in accordance with AJCC (2010) pathological staging criteria along with clinicopathological correlation and *BRAF* ^{V600E} mutation analysis by polymerase chain reaction was carried out. AJCC (2010) classification for cutaneous melanomas were applied to anal melanomas as they did not have any standard pathological staging criteria.

RESULTS: There were 39 cutaneous and 11 anal melanomas, both displaying earlier age at presentation (median: 51.5 years) and male preponderance. The most common invasive subtypes were acral lentiginous (cutaneous) and nodular (anal). Anal melanomas presented as thicker tumours (median: 7.8 mm). Presence of ulceration (88%), lymphovascular invasion (70%), perineural invasion (42%), satellite/in transit metastases (28%) and regression (16%) were noted. Six (42.9%) of 11 patients with nodal metastases displayed extracapsular extension. Metastases developed in 23

(46%) patients, the most common site being liver; five patients developed clinical recurrence. Majority of tumours were of stage T3&T4 (94%), N2&N3 (42%) and M1c (38%). Anal melanomas showed higher rates of recurrence and metastases with the propensity to develop metastases in multiple sites, especially on presentation. Increased frequency of metastases on diagnosis is associated with nodular melanomas (both anal and cutaneous) and melanomas exhibiting regression. Anal melanomas presented with thicker and ulcerated tumours and showed 2.4 times higher risk (95% CI: 1.4-4.1) for development of metastases as compared to cutaneous melanomas. Acral lentiginous melanomas were associated with elevated levels of serum LDH as compared to nodular melanomas which showed no difference in the serum LDH levels. Extranodal extension of tumour was associated with the presence of distant metastases.

The 1-year, 2-year and 3-year overall survival (OS) rates of all melanomas were 77.8%, 22.2% and 5.6% respectively with a median OS of 16 months (95% CI: 14-35 months). Lesser OS was significantly associated with medium cell size and involved peripheral margin of invasive component. The 1-year, 2-year and 3-year distant metastases free survival (DMFS) rates were 64.3%, 28.6% and 14.3% respectively (median: 25 months, 95% CI: 8-36 months). Presence of satellitosis and in transit metastases had a significant negative impact on the distant metastases free survival. At the end of one year, 40% patients were found to be free of recurrence (mean: 29.8 months, 95% CI: 22.5-37.1 months). Factors associated with poor prognosis included gender (females), ulceration, increased Clark level, vertical growth phase, absence of tumour infiltrating lymphocytes, increased mitotic rate (> 12/mm^2), amelanotic

tumours, involved margins, larger metastatic nodal size (> 6cm), elevated levels of serum LDH and advanced stage. These findings were not statistically significant. There was complete absence of *BRAF*^{V600E} mutation in all the amplifiable cases (n=45), with an estimated probable prevalence of 2.7% in our population. Increased nest formation and upward scatter, larger pigmented cells with epithelioid morphology and continuous lateral circumscription were associated with absent *BRAF*^{V600E} mutation in our population.

CONCLUSIONS: Primary melanomas, both cutaneous and anal, show earlier age at presentation as compared to Caucasian population. Acral lentiginous melanoma is the most common subtype, unlike Caucasians who have predominance of superficial spreading melanoma. Anal melanomas display higher recurrence and metastases rate, especially on presentation involving multiple sites, similar to global studies. Significant adverse prognostic factors for overall survival include medium cell size and involved peripheral margins. Presence of satellite/in transit metastases is significantly associated with increased metastases and reduced survival. Female gender and amelanotic tumours tend to have poor overall prognosis. Complete absence of *BRAF*^{V600E} mutation in all the amplifiable cases, with an estimated probable prevalence of 2.7% in our population shows that the actual prevalence of *BRAF*^{V600E} mutation in the Indian population is much lower than that described in Asian literature. Increased nest formation and upward scatter, larger pigmented cells with epithelioid morphology and continuous lateral circumscription are associated with absent *BRAF*^{V600E} mutation in our population.

KEYWORDS: Melanoma, Histomorphology, BRAF

ABBREVIATIONS

AJCC: American Joint Committee on Cancer

ALM: Acral lentiginous melanoma

DNA: Deoxyribonucleic acid

IFN: Interferon

LDH: Lactate dehydrogenase

NM: Nodular melanoma

PCR: Polymerase chain reaction

SEER: Surveillance, Epidemiology and End Results

SSM: Superficial spreading melanoma

UV: Ultraviolet

WHO: World Health Organisation

CONTENTS

INTRODUCTION

INTRODUCTION:

Malignant melanoma is one of the most aggressive and fatal forms of cancer. There is substantial variation in the worldwide incidence of melanoma. The current WHO classification of malignant melanoma includes four major subtypes: Superficial spreading melanoma (SSM), lentigo maligna melanoma (LMM), acral lentiginous melanoma (ALM) and nodular melanoma (NM). (1) The overall incidence of malignant melanoma in Asian population is low. (2) But, it has been observed that the Asians have a relatively higher incidence of acral lentiginous melanomas. (3) Recent literature has highlighted the distinct mutations occurring in melanomas arising from different anatomic sites based on the degree of exposure to sunlight, with an emphasis on its pathogenesis and the approaches to molecular classification. *BRAF* mutation is one of the extensively studied genetic alterations in cases of malignant melanoma. This progress in understanding the molecular pathogenesis of malignant melanoma has also facilitated the development and utilization of targeted drugs and new therapeutic approaches. These studies have also prompted researchers to form hypotheses correlating the *BRAF* mutation status to a gamut of defined histomorphological features. (4) Most of these studies have been conducted in the Caucasian population, with only a limited number of reports in the Asian population. We have carried out a retrospective observational study on primary cutaneous and anal malignant melanomas, with emphasis on the histomorphological features according to the recent AJCC (2010) classification, survival analysis, prevalence of $BRAF^{V600E}$ mutation using molecular methods and correlation between *BRAF* mutation status and clinicopathological features.

AIMS AND OBJECTIVES

AIMS:

This project aims at studying the histomorphological features, survival analysis and prevalence of *BRAF*^{V600E} mutation in primary cutaneous and anal malignant melanoma in Asian population at a tertiary care centre and also to determine the correlation of *BRAF* mutation status with clinicopathological features.

OBJECTIVES:

1) To carry out a retrospective complete histomorphological study on primary cutaneous and anal malignant melanoma diagnosed in Christian Medical College, Vellore between January 2013 and December 2015.

2) To analyze the *BRAF*^{V600E} mutation status of the above mentioned cases.

3) To correlate the *BRAF* mutation status with a set of defined histopathological parameters and relevant clinical features.

4) To correlate the clinicopathological features with local recurrence, metastases and survival outcome.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

Melanoma (Greek) – "Melas": Dark and "Oma": Tumour

Melanoma is derived from melanocytes, the pigment producing cells present in the skin. Malignant melanoma is one of the most aggressive forms of cancers and its incidence appears to increase rapidly with time. The worldwide incidence of cutaneous and anal malignant melanomas displays a substantial variation. Currently, the average rate of increase in the incidence is in the order of 4–6% per year. (5)

History:

Sir Robert Carswell, a renowned pathologist, coined the term "melanoma" in the year 1838. (6) But historically, the initial documented descriptions of melanoma appeared in the recordings of Hippocrates in the 5th century, B.C. Many references in the European medical literature to "fatal black tumours with metastases and black fluid in the body" are found in the writings of Highmore (1651), Bonet (1651), and Henrici and Nothnagel. Sir James Paget (1853) was the first to describe and document the transition of malignant melanoma from a radial growth phase to a vertical growth phase. (7)

Wallace Clark (1966) put forth a standard scaling system to assess the prognosis of melanoma based on histopathological examination, now known as the "Clark's levels". Alexander Breslow(1970) observed that prognosis of cutaneous melanoma was related to tumour size and level of infiltration with tumour thickness. These criteria aided in the stratification of patients for prophylactic lymph node dissection and currently, sentinel node biopsy. (8)

Epidemiology:

Current statistics reveal that the total number of newly diagnosed cases of cutaneous melanoma was 22.3 per 100,000 men and women per year and total number of deaths was 2.7 per 100,000 population per year. Based on the 2012-2014 data from SEER, about 2.2 percent of men and women will be diagnosed with cutaneous malignant melanoma at some point in their lifetime. (5) Majority of the cases (85%) occur in developed countries. The age adjusted incidence rate in India is one of the lowest, being 0.3 per 100,000 population. (2) But, it has been observed that the Asian population has a relatively higher incidence of acral lentiginous melanomas than the other subtypes. (3) Most of the cases present with early-stage disease, but a major proportion can also present with loco-regionally advanced or metastatic disease.

Anal melanomas are relatively rare and constitute only 1-2% of all cases of melanomas and 23.8% of all mucosal melanomas. While the cancer registries in the United States show an overall incidence of 1.7 cases of anal melanomas per 100,000 population per year, it is only about 1.19% in Asian population. (9,10) Anal melanomas also showed ethnic differences with the African-American or Hispanic races presenting with mucosal melanomas more commonly than melanomas occurring at other sites, exception being acral lentiginous subtype of cutaneous melanoma. (10) Majority of the lesions show increased tumour thickness at presentation. Overall incidence of anal melanomas is difficult to determine due to lack of studies with large cohorts.

Melanoma accounts for 1–3% of malignancies occurring in paediatric age group. (11) The overall mortality after atleast 5 years follow-up is 29% (12) Congenital melanoma through transplacental spread has also been documented in the literature. (13)

Etiopathogenesis:

Melanocytes are derived from precursor cells in the neural crest, from where they migrate towards the basal layer of the epidermis of skin. During this process of migration, melanocytes may localize in other epithelia in tissues such as uvea, meninges and ectodermal mucosa (as present in the anal region). Usually in normal skin, one melanocyte is present for every 4-10 basal keratinocytes, despite racial differences. Therefore, the number and size of melanosomes found in keratinocytes and melanocytes determines the skin colour. One of the main functions of melanocytes is to produce and secrete melanin, which offers protection against the harmful effects of ultraviolet irradiation. (14,15)

The most significant predisposing environmental factor is excessive exposure to ultraviolet (UV) light (16) resulting in a 1.7-fold increase in risk of developing cutaneous melanomas. (17) In 1956, Henry Lancaster made the first observation that the risk of developing melanoma, especially in Caucasian populations, was associated with ‘latitude’ or the intensity of exposure to sunlight (18) Intermittent heavy exposure to sunlight, especially ultraviolet-B (UV-B) has been noted as a major risk factor in the aetiology of melanoma. (19) Exceptions include acral lentiginous and mucosal subtypes of melanoma including anal melanoma, due to their anatomical locations being relatively less exposed to sunlight. Evidence also suggests UVA as an

influential factor in some cases. (20) Studies have also hypothesised about chronic pressure, trauma and physical stress being associated with acral lentiginous melanomas as they occur commonly in the weight bearing portions of the soles, thumbs and toes. (21,22)

Melanin pigment displays natural protective effect against the ill effects of solar radiation and therefore cutaneous malignant melanoma is rare in dark-skinned races. The pathogenesis of melanoma involves free-radical formation induced by UV radiation, and thereby resulting in the development of pyrimidine dimers. Patients who are deficient in the major damage repair mechanisms, such as xeroderma pigmentosum, display an increased risk of developing neoplasms arising from the skin, including melanoma. Intensive exposure to sunlight, such as multiple episodes of severe sunburn in childhood is associated with superficial spreading melanoma. Lentigo maligna melanoma is closely related to chronic lifelong exposure to sunlight. (23)

Melanoma may develop within a pre-existing benign melanocytic nevus, arise de novo, or occasionally, develop within a cellular blue nevus. The pre-existent benign nevus could be either congenital or acquired in origin. Approximately 35% of nodular and superficial spreading melanomas evolve from melanocytic nevi. (24) Giant ‘bathing trunk’ nevi, have a higher rate of malignant transformation of around 3-18% (25) Lentigo maligna melanoma does not arise from a pre-existing benign or dysplastic nevi.

Other independent risk factors include family history, previous history of melanoma, multiple atypical moles or dysplastic nevi, and a few inherited mutations, such as *CDKN2A*/*p16INK4A*. Familial melanoma accounts for 8-14% of the overall cases of malignant melanoma. (26,27) Genes implicated in the pathogenesis of melanoma include *CDKN2A (9p21)* and *CDK4 (12q14)*. *CDKN2A* encodes the protein p16, which normally functions to inhibit the activity of *CDK4*. (28) Germline mutations in *CDKN2A* are noted in 20–30% of patients with familial melanoma. (29) Affected patients are younger than those with sporadic melanoma and frequently develop multiple tumours. Dysplastic nevi are also more common in the familial category.

The etiopathology and risk factors for anal melanomas are not clearly elucidated. Exposure to ultraviolet radiation present in sunlight, one of the most important predisposing factors in cutaneous melanoma, does not appear to play a role in the pathogenesis of anal melanoma due to its anatomic location. There are a few genetic alterations such as loss of *c-KIT* expression which is postulated in the pathogenesis and aggressive clinical behaviour of anal melanoma. (30)

Clinical features:

Malignant melanoma presents more commonly in the sixth and seventh decades of life, the median age at diagnosis being 64 years and displays a male preponderance (3:2). Males also show an increased mortality rate when compared to females. (5,31) Females usually have thinner tumours at the initial period of diagnosis. Studies conducted in Asian population have shown earlier age at presentation, with a median

age at diagnosis of 56 years (21), while a study conducted in India showed a mean age at presentation of 49 years. (32) As noted globally, there is an overall male predilection for melanomas in Asian population as well. (32,33) The leg (particularly the calf) is the most commonly affected site in females, while the trunk and back are the most frequent sites in men. Involvement of head and neck region is also more common in males. Tumours developing on sites such as upper back, posterior aspect of arm, nape of neck and posterior aspect of scalp (also called the BANS sites) behave more aggressively than those occurring on the extremities. (34) The most commonly involved sites in Asian population are the palmoplantar sites, subungual region and limbs.(33,35) **(Fig. 1)**

The following are the important features to clinically predict a lesion as potentially early melanoma which was put forth by the New York University as 'ABCDE' criteria: (36)

- **A**symmetry
- Irregular **B**orders
- Variability in **C**olour/Pigmentation
- **D**iameter of the lesion (≥ 6 mm)
- **E**volution: Recently acquired changes with rapid growth rate

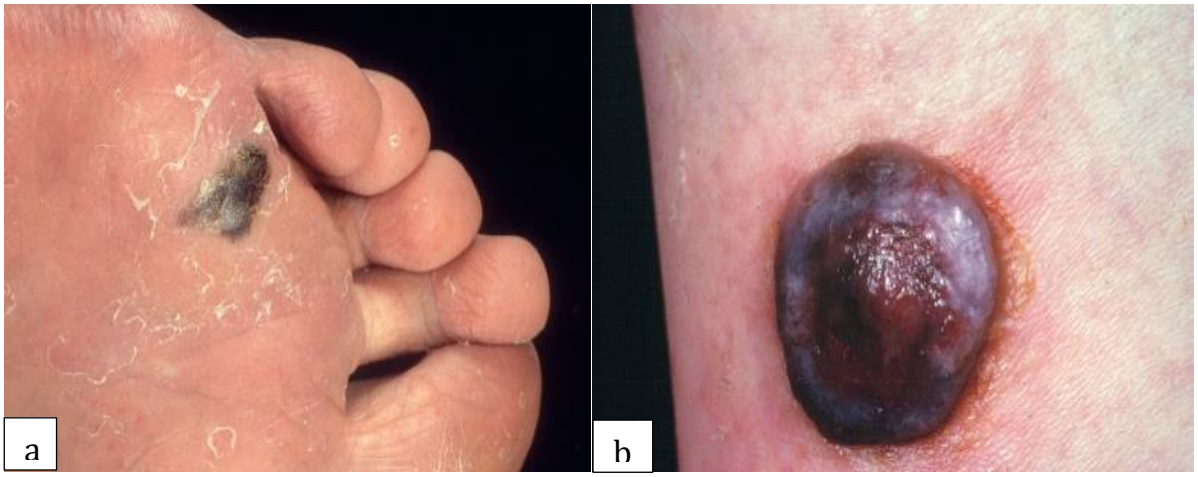


Figure 1. Clinical presentation of Acral lentiginous melanoma(a) and Nodular melanoma(b) *(Adapted from McKee's Pathology of Skin, 4th edition)*

Dermoscopic examination has been regarded as a valuable tool in the diagnosis of early melanoma. It is recommended as a diagnostic aid in all suspicious pigmented lesions. The features which are favorable for the diagnosis of melanoma include uneven thickness of the lines, presence of broad lines at the edge of the lesion, irregular distribution of black or brown dots in the lesion, bluish-white veil, atypical vascular pattern, and regression structures. Evidence indicates that the utilisation of dermoscopy, in addition to routine clinical examination increased the sensitivity in the detection of melanoma. (37)

Anal melanomas frequently present in the seventh decade, later than the usual presentation of cutaneous melanomas. (38) Studies conducted in Asian population have shown a wider age range of fifth to seventh decade. (39,40) Anal melanomas usually do not show any sex predilection, though few studies have indicated a slight female predominance. (41) In contrast, anal melanomas are more common in males in Asian population. (40) The common clinical features include bleeding, anal pain, anal

mass, pruritus and tenesmus. They present more commonly as an advanced stage disease, with increased tumour thickness. (10) Though there are reports of local spread of anal melanoma, they are usually not so aggressive as to infiltrate adjacent organs. They usual present as polypoid tumours, with or without pigmentation and ulceration. The diagnosis is usually made with visual inspection and/or by using anoscopy. Most common sites for metastases include inguinal and mesenteric lymph nodes, para-aortic lymph nodes, liver, lung and brain. (38) The overall survival time following diagnosis of anal melanoma is about 10-19 months. (42)

The presence of ulceration is regarded as an indication of a potentially poor prognosis in both cutaneous and anal melanomas. Clinical signs of nodule formation also signifies that the tumour has entered the phase of vertical growth.

Classification and histomorphology:

According to the current WHO classification (1), malignant melanoma is divided into four major subtypes, based on the histomorphological features:

- Superficial spreading melanoma (SSM)
- Lentigo maligna melanoma (LMM)
- Acral lentiginous melanoma (ALM)
- Nodular melanoma (NM)

The two main concepts which prove vital to this classification include the radial growth phase and the vertical growth phase. (43,44)

Radial growth phase: It is defined as presence of an in situ intraepidermal component with/without evidence of microinvasion into the papillary dermis, also known as the microinvasive radial growth phase. This phase lacks significant metastatic potential and shows an excellent prognosis. (45) There are single cells or small aggregates of tumour cells, histologically resembling their intraepidermal counterparts. These tumour cells form nests smaller than those seen within the epidermis. Presence of melanoma in situ spanning an area of 3 or more rete ridges beyond the invasive component (which is wider than deeper) is also one of the criteria for radial growth phase. (46) Mitotic activity is absent.

Vertical growth phase: It is characterised by the inherent ability of the melanoma cells to survive and proliferate within the dermis. There are cohesive nests, nodules or plaques composed of cells, which are more pleomorphic and mitotically active. These tumour nests and nodules are usually larger than their epidermal counterparts. Apoptosis is frequently present. This phase indicates a transformation with a potential for lymphovascular invasion and metastatic spread. (47) Majority of the tumours display a vertical growth phase accounting for 70-90% of the invasive melanomas. (48–50)

Superficial spreading melanoma:

Superficial spreading melanoma is the most common subtype of melanoma in the Caucasian population. It displays an equal sex predilection. (51) The most commonly affected sites include the leg (particularly in females) and the back (in males). It

usually presents as a flat scaly macule or plaque, which on development of invasion transforms into a blue or blue-black nodule. Amelanotic subtypes appear as erythematous or flesh coloured lesions. One of the characteristic features include scalloping seen at the lesion borders. Regression presenting as hypopigmented areas are also noted frequently. Ulceration may be present.

Superficial spreading melanoma is characterized by pagetoid spread of melanoma cells with large nuclei, distinct nucleoli and abundant pale cytoplasm. These atypical melanocytes may be distributed singly or form irregular nests. The tumour cells are present throughout all levels of the epidermis, referred to as "buckshot scatter". The invasive form displays one or more dermal aggregates, which appear larger than the largest cluster in the epidermis with or without the presence of mitotic activity. (52) Lack of maturation, characterised by failure of nest formation or the constituent cells, nuclei and nucleoli becoming smaller towards the base is noted. (1)

Lentigo maligna melanoma:

Lentigo maligna, also referred to as 'Hutchinson's melanotic freckle', characteristically develops on chronic sun-damaged skin in the elderly and displays a significant female predominance. (53) Sun damage to the skin is typified by epidermal atrophy and severe dermal solar elastosis (54) and accounts for 4% of all cases of melanoma occurring in the skin. Frequently affected sites include the head and neck region such as nose, temple and forehead. Acral sites may also be rarely involved. The lesion presents as a variably pigmented, gradually progressive, irregular and flat

macule. Hypopigmented areas representing regression may be present. Invasion usually occurs 10–15 years after the appearance of in situ lesion.

Lentigo maligna melanoma is characterized by a predominant junctional proliferation of atypical tumour cells forming irregular junctional nests. The atypical melanocytes are frequently seen to extend down the walls of appendageal structures such as hair follicles and sweat ducts forming junctional tumour nests. Multinucleate giant cells of the 'starburst' type are common. Dermal invasion in the form of atypical melanocytic spindle cells are frequently present. Invasion into the dermis from atypical cells present in the walls of hair follicles and sweat ducts are also common. (55)

Acral lentiginous melanoma:

Acral lentiginous melanoma accounts for 8–10% of all the cases of cutaneous melanomas. (56) It is the predominant variant affecting the dark skinned population of African-American and Asian origin, accounting for about 50-80% of all melanomas. (3,21,57) Sites of predilection include the non-hair bearing skin of palms and soles, with the heel being the most common site of occurrence. (22,58) Subungual variants of acral lentiginous melanoma frequently involve the great toe and thumb. It constitutes 2% of all cases of melanoma. Females are affected more often than males. These lesions most commonly present in the elderly population. (59) The lesions initially develop as irregular, enlarging and pigmented macules. The vertical growth phase is heralded by ulceration and development of blue or black nodular lesions. It is usually associated with a poor prognosis as tumours are most often thick at the time of

presentation. Mucosal melanomas were historically classified within the spectrum of acral lentiginous subtype, due to their partial morphologic overlap. Although acral lentiginous and mucosal subtypes of melanoma share a few similar molecular characteristics, they are considered to be distinct entities due to the differences in their clinical behaviour.

The radial growth phase in this subtype is characterized by marked acanthosis with elongated rete ridges, and lentiginous proliferation along the basal layer of epidermis. The atypical melanocytes display large bizarre nuclei, prominent nucleoli and abundant amounts of cytoplasm filled with melanin granules. They also show long, elaborate dendritic processes. The tumour nodules consist of predominantly spindle shaped cells in the vertical invasive growth phase, associated with a desmoplastic reaction and heavy band-like chronic inflammatory infiltrate.

Nodular melanoma:

Nodular melanoma accounts for 3–4% of melanomas and has a poor prognosis. Males are more frequently affected than females (2:1), and lesions usually appear in the fifth or sixth decade. (60) It has the propensity to involve the trunk and limbs. Those lesions which lack pigment are described as amelanotic melanoma. It is the second most common subtype accounting for about 12-20% of all melanomas occurring in Asian population. (3,35)

Nodular melanoma characteristically arises from vertical growth phase since inception. It usually presents as a raised, dome-shaped, or polypoid lesion, displaying

asymmetry. The atypical melanocytes present in the epidermis extend upto and not beyond three adjacent epidermal rete ridges. The dermal component is characterised by a cohesive nodule or small nests of atypical melanoma cells exhibiting a "pushing" or "expansile" growth pattern. The tumour cells show frequent cellular and nuclear enlargement, nuclear pleomorphism, hyperchromasia, and prominent nucleoli.

Other rare variants: (55)

Minimal deviation melanoma

It is a subset under invasive melanoma which exhibits only limited morphological deviation from the benign category of banal nevi and lack the pleomorphism and degree of atypia seen in classic malignant melanoma. (61–63) Subtypes include nevoid melanoma, small cell melanoma, and spitzoid melanoma.

Nevoid melanoma

Nevoid melanoma usually appears as verrucous to dome-shaped variably pigmented nevi, papules or nodules. Mitotic figures can be identified throughout the thickness of the tumour with mild pleomorphism. Evidence indicates a 50% recurrence rate, 25-50% metastasis rate and 25% mortality rate on follow-up. (64)

Small cell melanoma

Small cell melanoma is a high-grade melanoma, characterized by monomorphic population of small cells with scant cytoplasm, hyperchromatic nuclei and distinct nucleoli (resembling type-B nevus cells). Recent study has shown small cell

component in malignant melanoma as an independent indicator of poor prognosis. (65) In tumours where junctional activity is absent, and in metastatic disease, differentials include poorly differentiated or undifferentiated carcinoma, lymphoma and neuroendocrine carcinoma. (66) Immunohistochemistry can be utilised for an appropriate and accurate diagnosis.

Spitzoid melanoma

Spitz nevus is predominantly seen in children and young adults while malignant melanoma occurs frequently in the middle aged and elderly population. Malignant melanoma presenting in childhood displaying spitzoid features has been documented (67) and is one of the most difficult lesions to categorise. Features favouring the diagnosis of spitzoid melanoma are large size, penetration into deep dermis, atrophy of epidermis, pagetoid spread at the periphery of the lesion, asymmetry with lack of circumscription, absence of Kamino bodies, larger dermal nests or diffuse sheeting pattern in the dermis, lack of maturation, hyperchromasia, nuclear pleomorphism and deep and atypical mitosis.

Walsh et al have put forth a list of diagnostic criteria to distinguish Spitz nevus from spitzoid melanoma. (68) A grading system for classifying atypical Spitz nevi into low, medium, and high-risk categories was devised by Spatz et al. (69) Expression of HMB-45 in the deep dermis supports the diagnosis of spitzoid melanoma. (70) Studies also indicate that spitzoid melanomas lack mutations in *BRAF* and *NRAS* genes as opposed to other melanoma subtypes. (71) Recent evidence shows that these tumours

have the same risk of systemic spread and mortality rate as other subtypes of melanoma. (72)

Signet ring cell melanoma

Signet ring cell melanoma is a very rare histological subtype, most commonly described in metastatic tumours. Though there are recordings of signet ring cell features in recurrent and primary disease, it does not have a prognostic significance. (73–75) The appearance is ascribed to excess intermediate filaments appearing as intracytoplasmic vacuoles, which may be weakly positive with periodic acid-Schiff, diastase resistant but consistently negative with Alcian blue. It has to be distinguished from other tumours containing predominant signet ring cell change, such as mucin-producing adenocarcinoma and lymphoma.

Rhabdoid melanoma

The characteristic feature of rhabdoid melanoma is the histomorphological presence of eosinophilic hyaline intracytoplasmic inclusions. These inclusions are ultrastructurally identified as whorls of intermediate filaments. They are found to be tubular inclusions present within intracellular organelles like rough endoplasmic reticulum and mitochondria. (76,77) Currently, there are very few documented cases of rhabdoid phenotype in melanoma to assess any prognostic significance. The tumour cells failed to show S-100 and HMB-45 in some cases and the inclusions were found to be composed of keratin, smooth muscle actin or desmin. (76)

Balloon and clear cell melanoma

Balloon cell melanoma is a rare vertical growth phase histological subtype of malignant melanoma. (78) They display an increased tumour thickness at presentation and therefore is associated with poor prognosis. The tumour cells contain abundant eosinophilic or clear cytoplasm which exhibit fine granularity or vacuolation, containing periodic acid-Schiff (PAS) positive, diastase resistant granules. The vacuolation is ascribed to abnormal melanosome metabolism or degenerative processes occurring in melanosomes. (79) They must be distinguished from sebaceous and xanthomatous tumors, clear cell carcinomas and even liposarcoma.

Myxoid melanoma

Myxoid stromal change in malignant melanoma may present in primary, recurrent, and metastatic tumours (80,81) and also as a reactive change post phototherapy. (82) Myxoid change may be rarely seen in desmoplastic melanoma. It has to be distinguished from mucus-secreting adenocarcinoma and myxoid variant of malignant nerve sheath tumour. It carries no prognostic significance.

Melanoma with neuroendocrine differentiation

Melanomas displaying neuroendocrine differentiation have been rarely documented. (83) Characteristic melanocytic markers remain intact along with immunohistochemical expression of neuroendocrine markers such as synaptophysin and chromogranin.

Adenoid and pseudopapillary melanoma

These variants are characterised by extreme discohesion of tumour cells in the intraepidermal and invasive component. Such striking discohesion leads to a false impression of an acantholytic disease. Occasionally, pseudoglandular spaces are formed which are filled with mucin , resembling adenocarcinoma. (84)

Blue nevus-like melanoma (Malignant blue nevus)

This encompasses a spectrum of lesions which include melanoma developing in a background of cellular blue nevus, common blue nevus or nevi of Ito and Ota. De novo variants are also present. (85) This variant displays a male preponderance. It can occur in all age groups. The tumour cells frequently exhibit spindled cell morphology. Epithelioid cells and mixed variants are also known to occur occasionally. Scattered dendritic cells are commonly present and the lesion may show a geographic pattern of necrosis. It is associated with *GNAQ* mutation, a gene which encodes for the alpha subunit of heterotrimeric G-protein. (86)

Angiotropic and angiomatoid (pseudovascular) melanoma

Angiotropism has been documented in a few cases of melanoma. (87) It is characterized by the growth and spread of atypical melanocytes within or along the walls of blood vessels, particularly veins. This subtype usually does not display any intravascular invasion. The pericytic association in this variant is believed to represent a mechanism for local or regional metastasis. (88) Malignant melanoma may contain blood-filled telangiectatic spaces lined by atypical tumour cells resembling

angiosarcoma. Intraluminal tufting may also be seen resulting in the formation of pseudoglomeruloid structures. (89)

Metaplastic melanoma (melanoma with heterologous differentiation)

Melanoma is occasionally associated with the presence of heterologous metaplastic elements. These heterologous elements include components like bone and cartilage. (90) They are most often acral lentiginous lesions such as subungual melanomas. They are high grade tumours displaying considerable tumour thickness at the time of initial diagnosis with frequent metastases and increased rate of mortality. They appear as sarcomatoid tumours with osteoid, chondroblastic, smooth muscle and rhabdomyoblastic differentiation. (91) Cases of melanoma exhibiting ganglionic and ganglioneuroblastic differentiation have been described. (92) Exceptionally, melanoma may also contain multinucleated osteoclast-like giant cells. (93)

Pigment synthesizing melanoma (Animal-type melanoma)

Pigment synthesising melanoma, otherwise called melanoma with prominent pigment synthesis or low-grade hypermelanotic dermal melanoma is a rare subtype. The term "animal-type melanoma" was coined as these tumours were reminiscent of melanocytic lesions which usually occur in horses and other experimental animal models, thereby named as equine melanotic disease. (94) These melanomas most often occur in the second to fourth decades. This variant does not display any sex or site predilection. (95)

Histologically, the lesion shows a dense infiltrate of melanocytes exhibiting high pigmentation and is arranged in fascicles or nodules. These heavily pigmented tumour cells typically fill the papillary and reticular dermis and frequently infiltrate the underlying subcutaneous fat. The atypical cells have abundant fine to coarse melanin granules in the cytoplasm thereby obscuring the nuclear morphology. Mitotic activity is usually sparse. Though this variant of melanoma shows metastatic potential to involve lymph nodes, their prognosis is better than the traditional melanoma. (10)

Epidermotropic metastatic melanoma

Cutaneous deposits of melanoma may be associated with involvement of epidermis in a few cases such that distinguishing the metastases from a second or third primary tumour can prove to be an extremely difficult task, if clinical correlation is not undertaken. (96,97) Features favouring epidermotropic metastatic melanoma include a well-circumscribed nodule in the dermis, extensive lymphovascular invasion, and tumour infiltration of the papillary dermis associated with epidermal atrophy. (98) It is a satellite lesion resulting in increase in staging as per the AJCC staging system.

Desmoplastic and neurotropic melanoma

Desmoplastic and neurotropic subtypes of malignant melanoma are interrelated high-grade tumours. (99,100) Desmoplastic melanoma displays fibroblastic or myofibroblastic metaplasia associated with the synthesis of abundant collagen fibres. Neurotropic melanomas show Schwann cell-type differentiation. They are associated with a high recurrence rate (range 22–77%), metastasis(range 11–56%, mean 30%)

and a poor prognosis. (101) These tumours usually are seen in the elderly and display a male preponderance. Frequently affected sites are the head and neck followed by trunk and extremities. (102) Most often these tumours develop in a background of lentigo maligna variant of melanoma, typically on chronically sun-damaged skin. These lesions present as amelanotic, flesh-coloured, indurated and erythematous nodules. An important complication is spread along cranial nerves into the skull base followed by meningeal involvement resulting in an almost 100% mortality. Metastases to the lung is more common than regional lymph nodes. Factors favouring poor prognosis include increased mitotic rate, thickness of tumour and inadequate margins (less than1 cm) (103)

Desmoplastic melanoma is characterized by a spindled cell tumour with increased atypia, diffuse infiltration and marked fibrosis and collagenisation of the interstitium. Infiltration into the underlying skeletal muscle or bone is common. Frequently, the tumour is arranged in fascicles, but focal storiform areas may be seen occasionally. Lymphocytic infiltrates, commonly in the form of nodular aggregates, are a typical feature and an early histologic pointer of these lesions. (104) Neurotropic melanomas are tumours with marked nerve involvement resulting in clinical features of nerve irregularity and thickening. The tumour cells express immunohistochemical markers such as S-100 protein, neuron-specific enolase and often vimentin. HMB-45 may be positive in the tumor cells of the papillary dermis, but frequently it is negative. (105,106) Expression of smooth muscle actin is commonly present, reflecting the myofibroblastic population. Desmoplastic melanoma can be distinguished from

desmoplastic nevus by characteristic loss of p16 reactivity in the former group. (107) Differential diagnosis include non-neoplastic processes, such as scarring while deeply located lesions should be distinguished from fibromatosis and fibrosarcoma.

Lentiginous melanoma

Lentiginous melanoma has to be distinguished from the more common variant of acral lentiginous melanoma. Most frequently affected sites are the shoulders and upper back. (108) It is associated with an in situ phase for several years, described as atypical lentiginous melanocytic nevus and dysplastic atypical nevus of the elderly. (109) Lentiginous melanoma primarily comprises a single cell array of atypical melanocytes with very focal aggregation of these tumour cells resulting in the formation of junctional nests. The usual rete architecture at the dermo-epidermal junction is preserved. It is not associated with solar elastosis.

Primary dermal melanoma

The skin lesions in primary dermal melanoma presents as a dermal nodule. They do not have an in situ component and show no association with any pre-existing nevus. (110,111) It has a propensity for the head and neck region and extremities. Primary dermal melanoma is characterized histologically by a nodular deposit composed of atypical epithelioid to spindled cells located in the dermis and/or extending into the subcutis. It is extremely significant to distinguish this entity from metastatic melanoma, with regard to different staging implications. (112) Evidence indicates

origin from follicular melanocytes or complete regression of a previous intraepidermal component.

Melanoma in children

Malignant melanoma accounts for 1-3% of all malignancies occurring in childhood. (11) Congenital melanomas include those tumours which may develop in utero (within a giant congenital nevus or any de novo variant) or through transplacental spread (from melanoma in the mother). (13) But majority of the tumours are acquired and associated with exposure to sunlight during childhood. (113) Predisposing factors include genetic disorders such as xeroderma pigmentosum, dysplastic nevi, giant congenital nevi, family history of melanoma, previous history of irradiation, neurocutaneous melanosis (leptomeningeal melanoma) and immunodeficient status. (114,115) There is equal sex predilection with tumours occurring more commonly in the second decade. Paediatric melanomas behave no differently from those tumours occurring in the adults (116) and therefore require sentinel lymph node mapping and biopsy as recommended. (113)

Melanoma developing within a giant congenital nevus usually presents as a sharply delineated dermal nodule composed of tumour cells displaying cytological features of malignancy. Superficial spreading and nodular melanoma subtypes are most commonly noted. Other variants such as spitzoid melanoma, blue nevus-like melanoma and small cell melanoma must be diagnosed with caution. Tumour thickness and ulceration are the most important prognostic indicators.

Prognostic Factors:

The important clinical prognostic factors include age, sex and location of the primary tumour. (117,118) Older patients and males have a poorer prognosis. Acral sites are associated with a worse prognosis. (119)

The important variables to be documented in a routine histopathological reporting of melanoma include: (120,121)

- Ulceration
- Phase of tumour growth
- Breslow's tumour thickness
- Clark's level of invasion
- Mitotic rate
- Lymphovascular invasion
- Perineural invasion
- Tumour infiltrating lymphocytes
- Regression
- Microsatellite/ In transit metastases

The evaluation of tumour thickness by Breslow's method is the most important prognostic indicator. (122,123) The measurement of maximum tumour thickness is made using a calibrated ocular micrometer, and measured at right angle to the adjacent normal epidermis of skin. The thickness is measured from the granular cell layer (most superficial aspect) to the deepest point of tumour invasion. In cases with

ulceration, the measurement is made from the base of the ulcer (taken as the upper point of reference) till the maximum depth of invasion. Tumours of thickness 1.00mm or less were defined as thin melanomas, and were found to have excellent prognosis. (124,125) Few studies in the Caucasian population (126,127) have shown that 60-80% of the tumours have maximum tumour thickness $\leq$ 2.00 mm (range: 0.68-1.60 mm) while other studies show the average thickness ranging from 2.50-3.00 mm. (128,129) In contrast, Asians display a greater tumour thickness at presentation, with 30-45% of tumours having maximum thickness of $\geq$ 4.00 mm. (21,33) One Asian study has shown the average tumour thickness to be 8.27 $\pm$ 12.2 mm, taking into consideration cutaneous and non-cutaneous melanomas. (130)

The classification of tumour invasion based on Clark levels is as follows: (44)

- Level I: In situ (Intra epidermal) melanoma
- Level II: Invasion and expansion of the papillary dermis by single cells or small nests of atypical tumour cells
- Level III: Invasive tumour expanding and filling the papillary dermis and just abutting on the reticular dermal interface
- Level IV: Invasion of the reticular dermis
- Level V: Invasion of the subcutaneous fat

Though the Clark level was believed to be an independent prognostic indicator for thin tumours (1.00 mm or less in thickness), the 2010 (7th) AJCC staging system no longer recommends its utility, when a mitotic rate of the dermal component is

available. (123,131) Inspite of this recommendation, Clark level IV or V is still given significance as a tertiary criterion for T1b cases which do not display ulceration and when mitotic rate cannot be determined. Therefore, the upstaging of T1 lesions may be aided by the measurement of Clark level, when indicated. This distinction between T1a and T1b staging of tumours is of clinical significance, as biopsy and examination of sentinel lymph nodes is recommended for all malignant melanomas of stage T1b and above. (132) In keeping with greater tumour thickness in Asians, 45-70% of the cutaneous melanomas display Clark level of IV or more (50,133) while global studies have shown an increased frequency of Clark level III and IV. (128)

Ulceration is defined as the absence of an intact full thickness epidermis overlying a major portion of the primary melanoma based on microscopic examination of the histologic sections. Associated reactive changes such as fibrin deposition and neutrophilic infiltrate may also be present with thinning, effacement or hyperplasia of the adjacent epidermis. It is associated with a significant risk of metastasis. (134) Global studies have shown that 18-28% of cutaneous melanomas exhibit ulceration (128,129) while 60-65% of tumours in Asian population display ulceration. (33,35) Anal melanomas exhibit a higher frequency of ulceration ranging from 70-100%. (135,136) The presence of ulceration also predicts survival outcome in thicker (more than 1.00 mm) tumours. (137)

Tumour-infiltrating lymphocytes are an important prognostic indicator and classified as brisk, non-brisk or absent. (138) Brisk category includes lymphocytic infiltration

throughout the whole vertical growth phase. Non brisk indicates focal lymphocytic infiltration of tumour. Absent implies either no lymphocytes at all or scattered lymphocytes not infiltrating the melanoma. Both global and Asian studies have shown "non-brisk" to be the most common type of response. (127,133,139) Brisk lymphocytic infiltrates are commonly a feature of thin melanomas while absent lymphocytic response is usually seen in thicker melanomas and also correlate with metastasis to sentinel lymph nodes. (140)

Regression is an important feature evident in the dermal component and is particularly seen in thin melanomas. It is defined as a focal area with delicate and oedematous fibroplasia in the region of papillary dermis, often accompanied by telangiectasia and scattered melanophages and lymphocytes. Atypical melanoma cells are usually present in the epidermis and/or papillary dermis in either or both sides of the area of regression. (141) Regression is present in 26-48% of melanomas in Caucasian population (48,128) while it accounts for 20-25% of tumours in Asians. (133,142) Complete regression is an adverse prognostic indicator of invasive melanomas. (143) Studies recommend sentinel lymph node biopsies are indicated for thin melanomas if there is presence of extensive (>50%) regression. (117)

Mitotic rate is calculated as the number of mitotic figures per square mm of tumour in the most mitotically active area, known as "hot spot". (46) When the component of invasive tumour occupies an area of < 1 sq mm, the number of mitotic figures per square mm should be evaluated from the dermal tissue including the tumour. Tumours can also be classified as "mitogenic" and "non mitogenic" based on the presence

(atleast 1) or absence of mitotic figures in the invasive component if the tumour area is < 1 sq mm. Tumours with increased mitotic rate are associated with a poor prognosis. (48) Studies have shown majority of the tumours to be "mitogenic", with a mean value of 3.5/ mm^2 (48,130)

Satellites are discontinuous foci of tumour situated within 2 cm of the primary tumour mass. They could be macrosatellites or microsatellites. Microscopic satellite is defined as a distinct tumour nodule measuring 0.05 mm or more in diameter present in the reticular dermis, panniculus or blood vessels beneath the primary invasive tumour. This microscopic nodule is separated from the invasive tumour by at least 0.3 mm of normal tissue on the thickest part of tumour section. (46,141) It is associated with high rate of local recurrence, lymph node metastases, and decreased survival. (144) In transit metastases include skin or subcutaneous intra-lymphatic metastases situated more than 2 cm from the principle invasive tumour, but not beyond the regional nodal basin. (46) Clinically detected satellitosis and in transit metastases are present in 10-20% of patients with cutaneous melanomas. (145,146)

Lymphovascular invasion correlates with the presence of in-transit metastases (147) and appears to be a predictor of diminished survival. (148) Lymphovascular invasion is noted in 24% of cutaneous and non-cutaneous melanomas, with mucosal melanomas displaying a higher frequency of tumour emboli in vascular channels. (130,135,149) Perineural and intraneural infiltration are more commonly present in desmoplastic variants (150) and are associated with higher rate of local recurrence.

Presence of perineural invasion has been reported in 18-25% of all melanomas. (130,133,151) Angiogenesis, defined as development of new vascular channels at the base of the melanoma, is associated with tumour thickness, relapse and increased mortality. (152)

Management:

Surgical Excision:

The mainstay of treatment for primary malignant melanoma constitutes surgical excision. There have been many studies associating surgical excision margins with rate of local recurrence and survival outcomes, based on the tumour thickness. (153–155) Histopathological excision margin is the nearest radial margin of normal tissue adjacent to the excised tumour tissue. It is recorded by measuring the distance between the tumour and the labeled or inked lateral or deep margins under a microscope. (46) A correction factor of 20% is used to account for the shrinkage of tissue in the formalin fixed specimen, as compared to surgical excision margins. (156)

It has been proposed that melanoma in situ should have a excision margin of 0.5 to 1 cm. The surgical margin recommendations for invasive cutaneous melanomas with tumour thickness of $\leq$ 1.00 mm, 1.01-2.00 mm, 2.01-4.00 mm and > 4.00 mm are 1 cm, 1-2 cm, 2-3 cm and > 3 cm respectively in accordance with different National Guidelines. Narrower margins displayed a higher rate of local recurrence and higher mortality. (155,157,158)

In the case of anal melanomas, the recommended surgical excision margins are 1 cm and 2 cm for tumours with maximum thickness of $\leq$ 1.00 mm and 1.01-4.00 mm respectively. For tumours > 4.00 mm thick, abdominoperineal resection is recommended. In such cases atleast 1 cm histological surgical margin is recommended. (159,160) Nevertheless, a universal "safe minimum" margin has not yet been established. If a lateral margin is involved by either in situ or invasive tumour, the same should be reported as per AJCC recommendations. (46)

Lymph node dissection:

The clinical rationale for the identification and evaluation of sentinel lymph nodes is based on the hypothesis that metastatic involvement of a sentinel lymph node increases the probability of other distant lymph nodes containing metastatic tumour deposits. A sentinel node is the first lymph node to receive lymphatic drainage from any primary tumour. Sentinel lymph node biopsy provides extremely vital prognostic information and determines the probability of tumour recurrence and survival in stage I and stage II disease. (161) It is recommended that immunohistochemical markers like S-100, HMB-45 and/or MART -1 should be performed in those cases with initial morphologically negative sections. Sentinel node biopsy is currently recommended for all tumours measuring 1.00 mm or more in thickness, presence of ulceration, tumours with 50% or more regression and vertical growth phase melanomas.

Elective lymph node dissection is also being performed based on the hypothesis that metastatic tumour deposits from the primary tumour spread in a sequential manner to regional lymph nodes and then towards distant sites such as viscera and bone. Lymph

nodal involvement is present in 42% of cutaneous melanomas to about 85% in anal melanomas. (40,50) The risk of local relapse following lymph node dissection is about 15-20%. In those cases which display extranodal extension, involvement of 4 or more lymph nodes, cervical node involvement and recurrence, the risk of relapse is increased (30-50%). (162,163) Studies have shown the presence of extracapsular extension of tumour in lymph nodes in 25-66% of melanomas. (164,165)

Adjuvant therapy:

Adjuvant therapy, by definition, is provided after definitive surgical intervention has removed all clinically and histologically detectable tumour. It is offered with the purpose of reducing the risk of relapse due to occult disease. (163) It includes immunotherapy, chemotherapy, radiotherapy and isolated limb perfusion. The most important discovery in the field of immunotherapy is the utilization of Interferons (IFN). The mechanism of action behind IFN-α in melanoma is believed to be immunomodulatory in nature, with evidence suggesting significant impact on recurrence free survival as compared to overall survival. Other immunotherapeutic agents which are under clinical trials include the CTLA-4 blocking monoclonal antibodies (ipilimumab and tremelimumab) and anti-PD-1/PD-L1 monoclonal antibodies (pembrolizumab and nivolumab). There have been many studies based on the utilization of adjuvant chemotherapy following surgical resection in patients with high-risk melanomas. These include the use of agents such as megestrol acetate, vitamin A, vindesine and dacarbazine , either singly or in combination with BCG. Adjuvant radiotherapy may be useful particularly when patients show

intolerance towards high dose interferon therapy. Isolated limb perfusion was observed to have an impact on survival of patients with high risk primary melanoma. The proposed mechanisms include eliminating the occurrence of in-transit micrometastases and/or destroying the already established in transit metastases in the panniculus. (162,163,166) Adjuvant therapy was provided in only a minority of patients, accounting for 12-30% of all melanomas. (167,168)

AJCC Staging: (Tables 1-3)

Recent American Joint Committee on Cancer (AJCC) 7th edition (2010) pathological staging system of cutaneous malignant melanomas include the following sites: (132,169)

Eyelid, Penis and scrotum, Anal margin and perianal skin, Vulva, External ear, Skin of lip

Mucosal melanomas of head and neck, vaginal melanomas and conjunctival melanomas are excluded.

Primary tumour (T):

As per AJCC convention, "T" refers to a primary tumour which has not been treated previously. To evaluate pT, resection or excision of the primary tumour or biopsy adequate to accord the highest pT category must be taken into account.

Tx: Primary tumour cannot be assessed (e.g. following curettage or melanomas displaying marked regression)

T0: No evidence of primary tumour

Tis: Melanoma in situ

Table 1. AJCC 7th Edition (2010) Classification – T Stage

T classification	Thickness (Breslow thickness in mm)	Ulceration/Mitoses
T1	≤ 1.00	a: Without ulceration and mitosis < 1/ mm^2 b: With ulceration or mitoses ≥ 1/ mm^2
T2	1.01 - 2.00	a: Without ulceration b: With ulceration
T3	2.01 – 4.00	a: Without ulceration b: With ulceration
T4	> 4.00	a: Without ulceration b: With ulceration

Regional lymph nodes (N):

Nx: Regional nodes cannot be assessed (e.g. previous surgical removal for any other cause)

N0: No regional metastases detected

Table 2. AJCC 7th Edition (2010) Classification – N Stage

N classification	Number of metastatic nodes	Nodal metastatic mass
N1	1	a: Micrometastasis b: Macrometastasis
N2	2-3	a: Micrometastasis b: Macrometastasis c: In transit met(s)/ satellite(s) without metastatic lymph nodes
N3	4 or more metastatic/ matted lymph nodes	In transit met(s)/satellite(s) with metastatic lymph node(s)

Micrometastases (*Clinically occult*): Diagnosed after sentinel lymph node biopsy and completion lymphadenectomy (if performed).

Macrometastases: Clinically detectable nodal metastases confirmed by therapeutic lymphadenectomy or when any lymph node metastasis displays gross extracapsular extension of tumour.

Satellite lesions: Located within 2 cm of the primary tumour. They may be detected clinically (macrosatellites) or microscopically (microsatellites).

In transit metastases: Skin or subcutaneous metastases located > 2 cm from the primary tumour, but not beyond the regional nodal basin.

Distant metastasis (M):

As per AJCC convention, the designation "pM" is reported only when metastases have been documented by histopathological examination.

M0: No detectable evidence of distant metastases

Table 3. AJCC 7th Edition (2010) Classification – M Stage

M classification	Site	Serum LDH
M1a	Distant skin, subcutaneous or non-regional nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases Any distant metastases	Normal Elevated

Studies have shown that Asians usually present with stage III and stage IV tumours, a larger proportion being pathological stage of T4, in keeping with the greater thickness of tumour at diagnosis and increased frequency of ulceration. (133,146) Due to the aggressive behaviour of melanomas with involvement of multiple nodes, N2/N3 stages are more common in both Caucasian and Asian population. (50,165) Metastases were commonly detected clinically and/or with the help of radiological aids in many studies. Clinical stage M1c is observed in around 70% of the patients with metastatic melanomas (50), as the frequency of distant metastases ranged from 25-55% in both global and Asian studies. (130,165,170) Distant metastases was detected at presentation in 22-30% patients with anal melanomas, signifying more

aggressive behaviour and worser prognosis as compared to cutaneous melanomas. (159,171) Lung and liver are the most common sites of involvement. (172,173) The number of metastatic sites was found to have an impact on survival in recent literature, with mucosal melanomas presenting with metastases in multiple sites as compared to cutaneous melanomas. (173–175)

Serum LDH:

Serum levels of Lactate dehydrogenase (LDH) is considered as one of the important prognostic factors and thereby occupies a significant position in the staging of malignant melanoma. Elevated levels of serum LDH is associated with reduced overall survival and poor outcome in cutaneous and non-cutaneous melanomas, especially in patients with distant metastases. (168,176,177)

Survival Outcome:

Global studies have shown that the 5-year overall survival rates in cutaneous melanomas range from 55% to 83% (178,179) whereas in Asian population the overall survival rates are in the order of 10-50%. (21,35,146) Recurrence is noted in 20-30% cutaneous melanomas (3,180), with median recurrence free survival period of 25-47 months and 5-year survival rate of 60%. (3,181,182) The median distant metastases free survival period ranges from 9 to 28 months, with the 1-year, 2-year, 3-year and 5-year survival rates being 47%, 33%, 21% and 11% respectively. (172,173) Anal melanomas behave aggressively with a far reduced 5-year overall survival rate of 9-15% both in Asian and Caucasian population (41,183), with a median overall

survival of 6-22 months. (40,135,160) Recurrence rate is 59% with a median recurrence free survival period of 23 months. (135) The median time period for the overall survival of cutaneous and non-cutaneous melanomas in Asian population is 37 months (range: 0-119 months) with the 1-year and 5-year overall survival rates being 74.3% and 35.9% respectively. The median recurrence free survival period is 48 months (1-132 months), with the 1-year and 5-year survival rates being 69.3% and 44.2% respectively. (33)

AJCC Eighth Edition:

The new Eighth edition of AJCC has released the Cancer Staging Manual with a few modifications in the staging protocol for cutaneous melanomas. (184) **(Tables 6-8)**
A study conducted by Haydu et al has estimated the conditional survival of stage III melanoma patients. Majority of tumours were of pathological stage T3 (28.2%) and N1 (66%) according to the new 8th edition of AJCC staging of melanomas. The 1-year, 2-year, 3-year and 5-year conditional survival rates were in the order of 80.5%, 61.5%, 48.6% and 32% respectively. (185)

Molecular classification of melanomas:

Ultraviolet rays are believed to produce a wide range of mutations, the most common point mutations being C > T or CC > TT transitions, referred to as the "UV signature mutations". (186) But the most common mutations in malignant melanoma occurring in the oncogenes *BRAF* and *NRAS* do not represent the classical signature. Mutation at *BRAF* codon 600 is the most frequently observed change resulting in T to A transition.

(187) Mechanisms by which the cells acquire a selective advantage for survival include specific alterations in the sequences of the amino acid present in *BRAF* and *NRAS* culminating in their state of constitutive activation. However, critical tumour suppressor genes in the background of melanomagenesis, such as *CDKN2A*, *p53*, and *PTEN* present with UV signature mutations. (188,189) Ultraviolet radiation is believed to be only one of the causative factors in the etiopathogenesis of malignant melanoma. An example to emphasise this point is that melanoma can also develop on those anatomic locations which are relatively or completely protected from sunlight exposure such as palms, soles, nail beds and mucosal membranes. The absolute incidence rate of malignant melanoma arising in acral and mucosal sites is found to be relatively constant across the world. (190) *BRAF* mutation in melanoma is independent of exposure to ultraviolet radiation. *BRAF* mutations identical to those occurring in melanoma are also commonly seen in thyroid and colorectal cancers. (191)

The differences in the clinical and histomorphological presentation of melanomas based on the anatomical site and exposure to UV radiation resulted in the classic 'histogenetic' variants of melanoma proposed in the Sydney Classification. (192) Recent evidence indicates that a few mutations occurring in oncogenes and chromosomal aberrations correlate with specific clinical and histomorphological features of melanoma. This supports the hypothesis that malignant melanoma shows biologically distinct subclasses, which however correlate with the variants proposed in the original classification. Investigations to define the various subtypes of melanoma by integrating the genetic alterations with specific clinical and/or histopathologic

features, as well as other predisposing environmental factors is the current basis of research.

Melanomas on chronically sun-exposed skin

The pattern of exposure to sunlight (intermittent versus chronic) and the cumulative amount of UV radiation correlates with specific clinical and histopathologic presentations of cutaneous melanoma. Melanomas arising on the head and neck sites tend to occur in chronically sun damaged skin of the elderly, with a peak around eighth decade of life. (193) The histopathological signs of chronic sun damage are indicated by marked solar elastosis and actinic keratosis. Melanomas developing on the trunk display a peak incidence around fifth decade. They characteristically do not show evidence of chronic sunlight induced damage in the surrounding skin, but show more melanocytic nevi thereby indicating a common etiopathogenesis. (194) This highlights the significance of the duration, dosage as well as timing of exposure to UV radiation.

Molecular studies favour the concept of distinct melanoma subtypes based on the pattern of sunlight exposure. (195) Mutations in *BRAF* are most commonly (about 70%) found in non-CSD (chronic sun damaged) melanomas, whereas they show relatively lesser expression in CSD melanomas (around 15%). (196) The association of higher frequency of *BRAF* mutations with presence of melanocytic nevi and non-CSD melanomas supports the etiopathogenesis with respect to timing of exposure to UV radiation. Mutations or DNA copy number increases in the *KIT* gene occurs in

30% cases of CSD melanomas. (197) CSD and non-CSD melanomas also display significant differences with regard to the pattern of chromosomal aberrations. About 40% cases of non-CSD melanomas display losses of chromosome 10. *PTEN* gene on chromosome 10, encodes a negative regulator of the Phospho-inositol 3-kinase (PI3 kinase) pathway and is the most frequently affected region. Mutations in *BRAF* only activate the MAP kinase pathway and therefore might require a concurrent *PTEN* loss. Whereas, both MAP kinase and PI3-kinase pathways are activated by *NRAS* mutations, thereby obviating the necessity for *PTEN* loss. (198) Studies show that a few melanomas contain point mutations in the gene encoding PI3-kinase protein, predominantly in the catalytic subunit. (199) **(Fig. 2)**

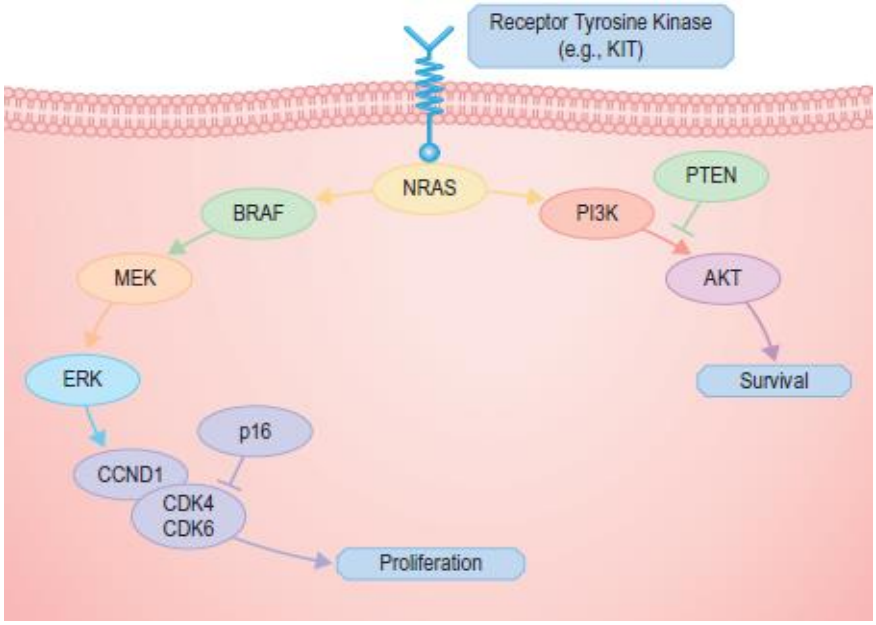


Figure 2. Schematic diagram representing important molecular pathways in melanoma *(Adapted from McKee's Pathology of Skin, 4th edition)*

BRAF is a serine/threonine protein kinase which is activated by the RAS-GTP protein and situated on chromosome 7q. It is one of the three isoforms of RAF (Rapidly Accelerated Fibrosarcoma) family of genes, present in the mammals. The mitogen-activated protein kinase (MAPK) pathway (RAS/RAF/MEK/ERK) is a critical

pathway in the pathogenesis of melanoma. (200) The most common *BRAF* mutation in cutaneous melanoma is T1799A transversion mutation occurring in exon 15 of the *BRAF* gene, resulting in V600E (Val600Glu) amino acid substitution in the corresponding protein. **(Fig. 3)** This point mutation constitutes over 90% of all *BRAF* mutations detected in cases of melanomas of skin. (187) The mechanism of action behind *BRAF* mutation is the sequential induction of constitutive extracellular signal-regulated kinase (MAPK/ERK) pathway. This growth pathway is involved in promoting the proliferation, survival and development of tumour cells. The overall frequency of *BRAF* mutation reported in primary melanomas is about 20-80%. (170,201–203) The prevalence of *BRAF* mutation in melanoma in situ is only around 5.6%, which is relatively less as compared to invasive melanomas. This emphasizes the association of *BRAF* mutation with progression rather than initiation in the natural course of human melanomas. (204) The prevalence of *BRAF* mutation in melanomas in Asian population is observed to be around 10-40% (205–209)

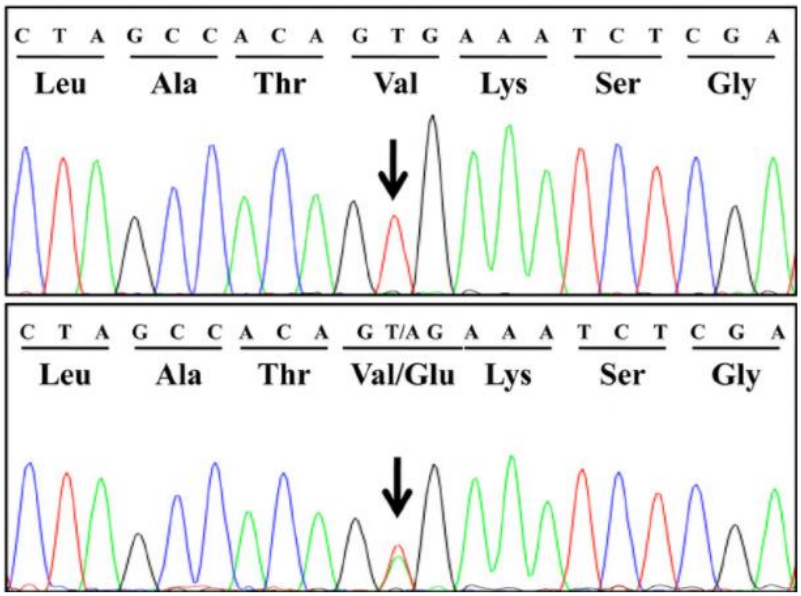


Figure 3. Sequence analysis of *BRAF* gene depicting *BRAF wild type* (a) and $BRAF^{V600E}$ mutation (b) *(Adapted from BRAF mutations in canine cancers, Mochizuki et al, 2015)*

The most common chromosomal aberration found in melanomas arising from non-chronically sun damaged skin are the gains in chromosome 7 (210) , which in turn is attributed to the increased frequency of *BRAF* mutation in such melanomas. Whereas about 50% of CSD melanomas show increase in copy numbers of the gene *CYCD1* located at chromosome 11q13, which encode the protein cyclinD1. Evidence also shows that there is an increase in p53 expression in cases of CSD melanomas. (211) Research shows that cutaneous melanomas display activation of the major proliferative and survival pathways. This may involve activating mutations in an upstream pathway, like the *KIT* gene, which encodes a tyrosine kinase receptor, or the downstream pathway involving *NRAS*. (212)

There have been various studies analysing and correlating the various histomorphologic aspects of the tumour in detail with the different mutations described in melanomas. Characteristically, melanomas which are known to have *BRAF* mutations display increase in the upward scatter of intraepidermal melanocytes, prominent nesting pattern, sharper lateral circumscription of the adjacent epidermis, thickening or hyperplasia of the involved epidermis and larger, rounder and heavily pigmented tumour cells. In a study by Viros et al, histomorphological features such as upward scatter, degree of formation of nests and cellular pigmentation were found to predict mutations in *BRAF* gene with up to 80% accuracy. (4) It was evident that *BRAF* mutation was highly unlikely in cases where the intraepidermal melanocytes show total absence of upward scatter.

It has been found that melanomas arising in chronically sun damaged skin as well as acral and mucosal sites show recurrent mutations in *KIT* gene which correlate histomorphologically with a lentiginous growth pattern and poor lateral circumscription. *KIT* plays a vital role in migration of melanoblasts from the neural crest and in the homing of melanoblasts into epithelial structures during development. Mutations which constitutively activate the *KIT* gene induces a migratory phenotype which accounts for the lentiginous growth pattern. (213)

Aberrant *KIT* signalling in melanomas explains the phenomenon of "field effect", which are frequently described in acral melanomas. (214) The 'field cells' are defined as those altered melanocytes in the histopathologically normal-appearing skin adjacent to the in situ portion of acral melanomas. They represent clonal expansions of melanocytes that carry at least part of the genetic alterations which are present in the adjacent invasive component, with implications of such cells representing precursors of the preinvasive melanoma in situ lesions, detected histopathologically. Studies indicate that the extent of these "field cells" beyond the histomorphologically identifiable in situ component can even be 1cm or more, nevertheless this extent does not display any association with tumour thickness. (215) However, this entity is of clinical significance, as it provides an objective guidance for the determination of radial margins and depth of excision during surgical removal, as part of the treatment protocol for primary cutaneous melanomas.

These studies indicate the potential for the development of classification schemes which could distinctly define biologically homogeneous subtypes of melanomas, and in turn leading to the drafting of various targeted treatment protocols based on the different distinct genetic alterations. **(Fig. 4)**

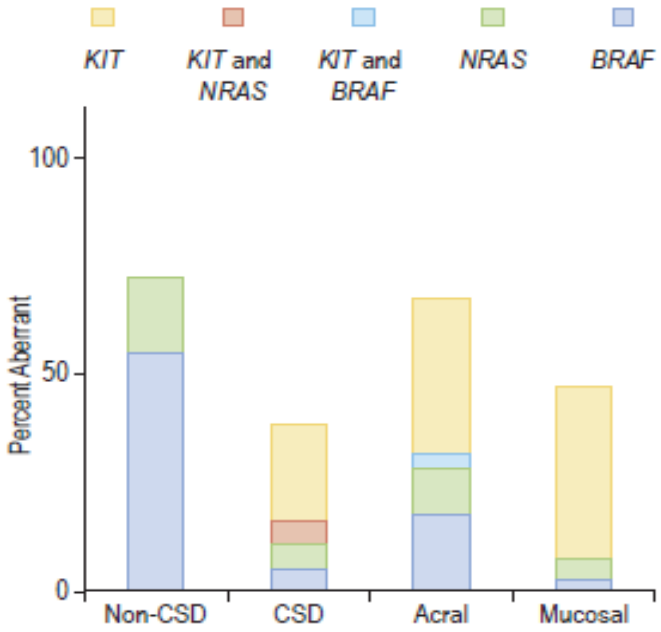


Figure 4. Frequency distribution of genetic alterations in different types of melanomas; CSD – Chronic sun damage *(Adapted from McKee's Pathology of Skin, 4th edition)*

Other genetic alterations described in malignant melanoma include *MITF* gene mutations or amplifications and mutations in beta-catenin. *MITF* is a transcription factor that has a vital role in the development of melanocytes derived from neural crest and retinal pigment epithelial cells originating from the region of optic cup. (216) Mutations in *CTNNB1* which encodes beta-catenin is a critical signalling constituent of the WNT pathway are also described in a subset of melanomas. (217)

Melanomas on non sun-exposed sites

Melanomas developing in acral sites, such as volar surfaces of the distal extremities are relatively protected from exposure to sunlight. The glabrous skin also displays a thicker stratum corneum which accounts for additional protection from ultraviolet radiation. Mucosal melanomas, as the name suggests, arise from the mucosal membranes which are completely unexposed to sunlight or ultraviolet radiation, the only exceptions being bulbar conjunctiva and lip. Evidence from genomic analyses indicate a higher frequency of chromosomal aberrations with consequent genomic instability occurring in acral and mucosal melanomas. (218) In contrast to the usual occurrence of such genomic alterations in the late phase of a disease, they arise early in the course of melanomas occurring in acral sites, sometimes even at the in situ stage. (214) This indicates the differences in the molecular pathogenesis in melanomas developing in sun-exposed skin and other unexposed sites. In melanomas occurring in sites with heavy exposure to sunlight, the by-products of ultraviolet radiation and consequent oxidative damage appear to be the significant insult, while double-stranded breaks in DNA appear to be the predominant mechanism of injury in acral and mucosal melanomas.

The most frequent genetic alteration seen in acral melanomas (about 50%) are amplifications in the *CYCD1* locus on chromosome 11q13. Whereas mucosal melanomas do not harbour genetic alterations of *CYCD1*, but show amplifications in the *CDK4* gene, which encodes cyclin dependent kinase 4, the binding partner of

cyclin D1. (210) *CDK4* is normally inhibited by p16 protein which acts as the gatekeeper of the G1/S transition point in the cell cycle.

Melanomas without association to epithelial structures

Most of the neoplasms originating from melanocytes are located within epithelial structures and harbour mutations in genes like *BRAF*, *NRAS*, and *KIT*. The subtypes of melanocytic tumours developing without any association to epithelial structures, however, do not carry the same genetic alterations. For instance, uveal melanoma, which originates from melanocytes situated in the choroidal plexus, iris and ciliary body display losses of chromosome 3 commonly, and show higher propensity for liver metastasis. (219) Another interesting fact in the pathology of uveal melanoma is the strong expression of *KIT* on immunohistochemistry, whilst lacking *KIT* gene mutations.

Studies indicate germline mutations in *GNAQ* and *GNA11*, the heterotrimeric G-protein subunits induce intradermal melanocytic proliferation resulting in hyperpigmented skin lesions. (86) Recent studies show somatic mutations of *GNAQ* gene in blue nevi and uveal melanomas. (220) Activation of *GNAQ* results in release of diacylglycerol (DAG) by phospholipase C and consequent activation of protein kinase C family. $GNAQ^{Q209L}$ mutation has also been found to activate the MAP-kinase pathway. Loss-of-function mutations of *PRKARIA* gene encoding a regulatory subunit of the protein kinase A complex have also been described with subsequent *MITF* activation.

Molecular therapy:

Due to the significant survival benefits achieved from MAPK-pathway targeted therapy in cases of advanced stage malignant melanomas, US FDA has recently approved *BRAF* inhibitors vemurafenib and dabrafenib, and MEK inhibitor trametinib. Trametinib is a MEK1/2 inhibitor with studies showing survival benefits in patients with metastatic melanoma. Drug resistance is one of the inevitable disadvantages emerging within a short period of time following initiation of treatment. This could be overcome by targeting different types of molecules in the same signaling pathway, such as combination of a *BRAF* inhibitor and a MEK inhibitor, which in turn would delay acquired resistance, prevent development of secondary malignancies, thereby enhancing tumour eradication. (166,221)

Genetic analyses have revealed mutations occurring in different pathways, either at the level of receptor (*KIT*) or downstream pathways (*NRAS*, *PTEN*, *BRAF*, *PI3KCA*). Specific mutations have found to correlate with anatomic location of the primary melanoma, as well as distinct clinical and histopathological features. Next generation sequencing based tools of genetic analyses have definitely yielded significant data to devise a comprehensive classification for the definitive diagnosis of primary cutaneous malignant melanoma and its subsequent management.

JUSTIFICATION

JUSTIFICATION FOR THIS STUDY:

Malignant melanoma is a rare disease with one of the lowest incidences occurring in Asian continent. (2) Therefore knowledge and information regarding the clinical and pathological characteristics and survival outcome in Asian population is limited. There are very few studies from India detailing the histomorphological features with clinicopathological correlation and survival analysis in primary cutaneous and anal malignant melanoma. (32,222–224) To the best of our knowledge following extensive review of literature, ours is the first study in India to analyse and report on the status of $BRAF^{V600E}$ mutation in primary cutaneous and anal melanomas.

MATERIALS AND METHODS

MATERIALS AND METHODS:

This project was approved by the Institutional Review Board (Ref: IRB Min. No. 9827 dated 07/01/2016) and funding provided under the Fluid Research Grant.

This is an observational study (cohort study) carried out in the Department of Pathology and Molecular Pathology laboratory in Christian Medical College (CMC), Vellore. Cases of primary cutaneous and anal malignant melanoma diagnosed in CMC Vellore from January 2013 to December 2015 were selected and the clinical details for the corresponding cases were collected from the Electronic Medical Records of our Institution. The formalin fixed paraffin embedded tissue blocks and slides of all specimen types (obtained between January 2013 and December 2015) including biopsies, resections and/or slide and block referrals of all these cases were retrieved from the archives of the Department of Pathology. Each block was assessed for the adequacy of tissue for the purpose of DNA extraction and molecular analysis.

Inclusion criteria:

1) Cases of primary cutaneous malignant melanoma, which include the following sites:

- Anal margin and perianal skin
- Vulva
- External ear
- Skin of lip

2) Anal melanoma

3) Adequate tissue for molecular analysis

Exclusion criteria:

1) Mucosal melanomas of head and neck, vaginal melanomas and conjunctival melanomas

2) Melanomas of eyelid, penis and scrotum

3) Absent/ Insufficient tissue for molecular analysis

Following selection and retrieval of all the cases, a complete histomorphological study, in accordance with AJCC (2010) pathological staging criteria and correlation of these histopathological parameters with clinical features including local recurrence, metastases and survival was carried out. As anal melanomas did not have any standard pathological staging criteria, AJCC (2010) classification for cutaneous melanomas were utilised to classify those cases as most of the lesions were in close proximity to the anal margin. (9,225) Tumour thickness/depth was given significance to clinically classify and stage anal melanomas according to the AJCC criteria. (225,226) All cases were subjected to $BRAF^{V600E}$ mutation analysis by Polymerase chain reaction (PCR), as standardized by our Molecular Laboratory and prevalence of the *BRAF* mutation was determined. Follow-up of patients was determined verbally by personal communication (whether dead or alive) and also from electronic health records, and the duration was taken from the first outpatient visit till the point of death or until the last follow-up date, as the case may be.

Variables:

- Clinical details: Age, gender, anatomic site of involvement, satellite/ in transit metastases, maximum size of tumour and lymph nodes, adjuvant treatment, serum LDH levels, local recurrence, metastases and survival outcome
- Histopathological features: Ulceration, Breslow's tumour thickness, Clark's level of invasion, mitotic rate, growth phase of tumour, lymphovascular invasion, perineural invasion, tumour infiltrating lymphocytes, regression, surgical resection margins, extranodal extension of tumour
- Degree of sun exposure and sunlight induced damage
- Additional histomorphological parameters: Upward scatter and nesting pattern of intraepidermal melanocytes, pigmentation, epidermal contour, lateral circumscription, size and shape of cells, nuclei and nucleoli
- Molecular analysis: $BRAF^{V600E}$ mutation status

Data Measurement:

All standard histopathological parameters were measured according to the definitions referenced by recent WHO Classification and AJCC (2010) pathological staging classification. (1,123) Breslow's thickness and mitotic index per mm^2 was calculated accurately using a crossed scale reticule for Olympus CX31.

Degree of exposure to sunlight and induced damage was assessed by presence or absence of marked solar elastosis.

Additional histopathological parameters, such as upward scatter and nesting pattern of intraepidermal melanocytes, pigmentation, epidermal contour, lateral circumscription, size and shape of cells, size of nuclei and nucleoli were measured as per the definitions provided by Viros et al, in a study correlating the histomorphological parameters and *BRAF* mutation status. (4)

Histological peripheral excision margins were classified into different categories based on recommendations from different National Guidelines for cutaneous melanomas. (155,157,158) The same classification was applied to the deep excision margin as there were no separate guidelines or recommendations applicable for deep margins. Excision margins for anal melanomas were also classified based on tumour thickness. (159,227)

Anal melanomas were classified according to the AJCC (2010) pathological classification as they did not have any standard pathological staging criteria. (9,225)

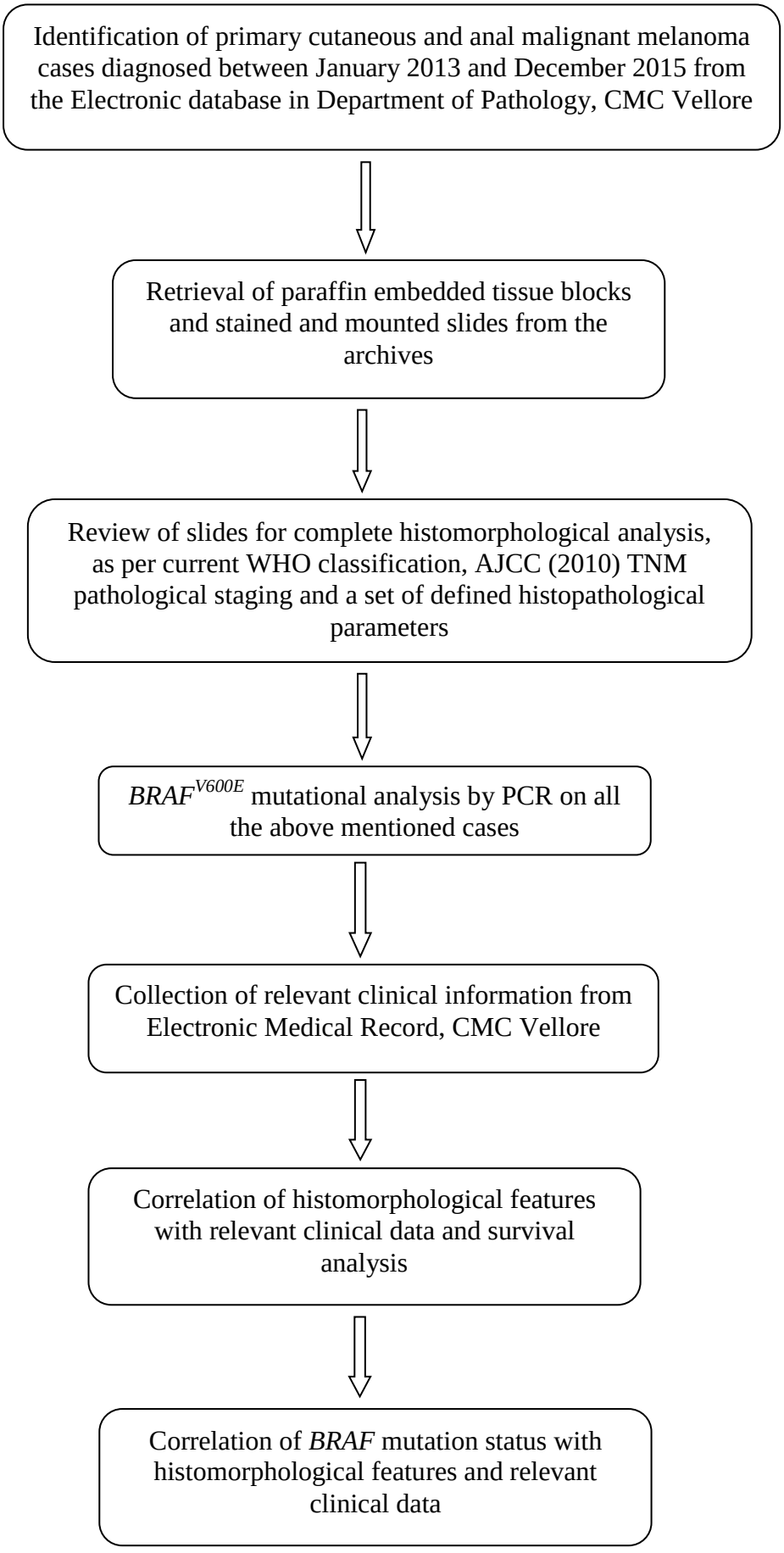
For the purpose of effective statistical univariate and multivariate analysis, most of the histomorphological data were taken into account from specimens of excision or resection, where available. The size or thickness of tumour in each case was taken from those specimens which had the maximum measurement, regardless of their type, so that the maximum pT value could be evaluated. (46)

Local recurrence was defined as a tumour growth within 2 cm of the primary site of surgical scar or graft after a disease free interval. (228) Recurrence free survival (RFS) was defined as the time period between the definite surgical intervention and the diagnosis of first local recurrence. Distant metastases free survival (DMFS) was defined as the time period between the initial histological diagnosis and the clinical diagnosis of distant metastasis. Overall survival (OS) was defined as the time period between the first outpatient visit and death or date of last follow up, as the case may be.

***BRAF* mutation analysis: (Refer Annexure 2)**

Formalin fixed paraffin embedded tissue blocks were used for DNA extraction and the extracted samples were checked for their quality and quantity prior to molecular analysis by Polymerase chain reaction (PCR). The PCR product was detected by agarose gel electrophoresis, following which sequencing was performed. Mutational analysis was carried out by comparing the sequence with the wild type and by looking for the presence of all known mutations in this exon.

Detailed diagrammatic algorithm of the study:



Elimination of bias:

To eliminate the occurrence of bias in this study, the review of histomorphological features in all cases was performed by at least 2 investigators (**NTA** and **MT**). A single protocol was applied to all samples by standardizing the PCR process in the molecular laboratory. All the histomorphological features were reviewed prior to analyzing the *BRAF* mutation status.

Calculation of Sample size:

With an expected prevalence of *BRAF* mutation of about 25% in primary malignant melanoma (205) and a precision value of 15%, the minimum requirement of number of subjects was given by the formula (using nMaster Version 2.0),

$$n = 4p(1-p) / d^2 = 32 \qquad \text{(where, } p=0.25 \text{ and } d=0.15\text{)}$$

However, we collected all cases of primary cutaneous and anal malignant melanoma diagnosed in CMC Vellore, between January 2013 and December 2015.

Statistical Analysis:

The data was entered in the data entry form (Epidata Version 3.1) and summarised using mean(SD) and frequency (percentage). Fisher's Exact/ Chi-Square test was used based on total number for categorical comparisons for events like recurrence, overall metastases, distant metastases and death. Kaplan-Meier curves were presented with mean survival (95%CI), and Log rank test was used to compare the equity of survivors among other categorical variables. Relative risk (95% CI) was presented to show the risk of variables over metastases, based on Breslow thickness. All the analysis were performed using STATA I/C software (Version 13.1).

RESULTS

RESULTS:

Fifty cases of primary malignant melanoma, including 39 cases of cutaneous melanoma (78%) and 11 cases of anal melanoma (22%) were diagnosed in our institution between January 2013 and December 2015. We had a total of 74 surgical specimens from all the above mentioned 50 cases, of which two were biopsies for tumour recurrence and therefore were excluded from the main analysis. Seventy two surgical specimens **(Table 9)** were studied in detail for the histomorphological features. Out of 50 patients, 22 patients had only biopsies, 7 patients underwent resection directly and 21 patients had undergone excision or resection following preliminary biopsy or slide and block review. One case with invasive melanoma in the initial biopsy showed only in situ lesion of the epithelium in the resection specimen.

The median age of the patients with primary malignant melanoma was 51.5 years (20-114 years), with that of cutaneous and anal melanomas being 56 years (28-114 years) and 48 years (20-57 years) respectively. **(Fig. 5)** There was an overall male preponderance (M:F ratio of 1.7:1), with cutaneous melanomas having M:F ratio of 1.6:1 and anal melanomas displaying M:F ratio of 2.6:1. **(Fig. 56)** The most common site involved by melanoma was "foot" (especially the heel region) constituting about 79.5% of all cutaneous cases. **(Fig. 6)** Almost all the patients were from the southern, eastern and central regions of India, with the exception of one patient, who came from Bangladesh. **(Table 10)**

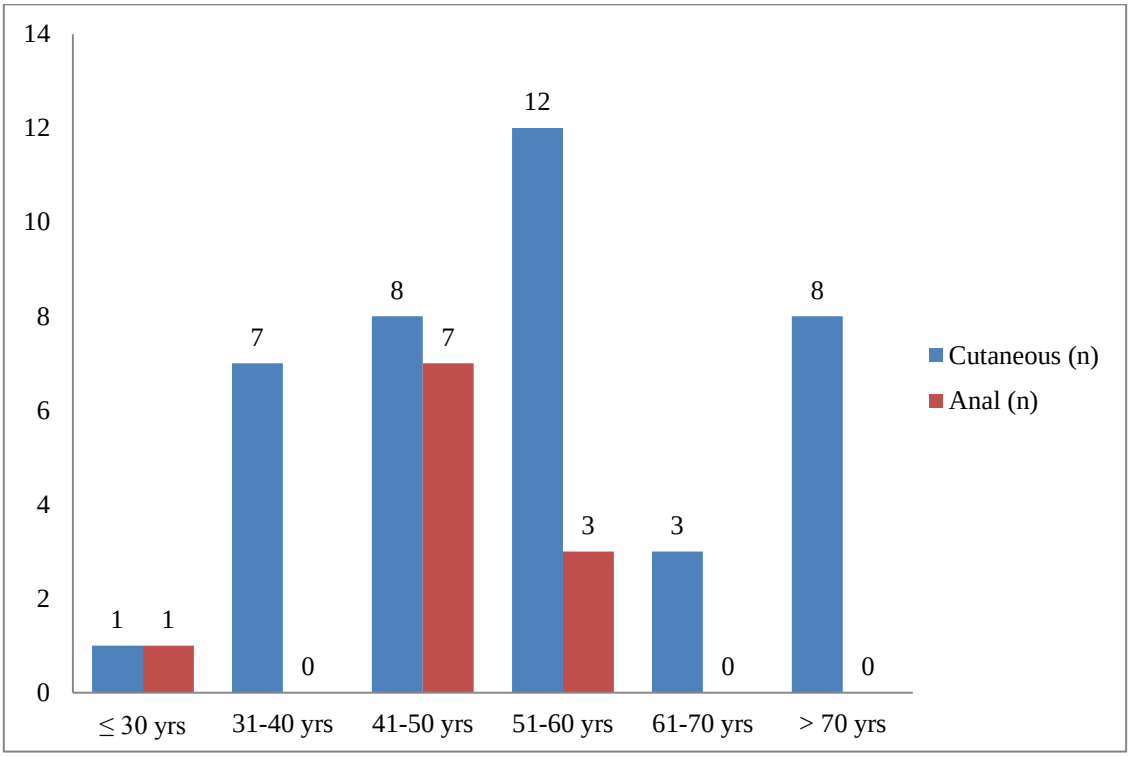


Figure 5. Age distribution in melanoma

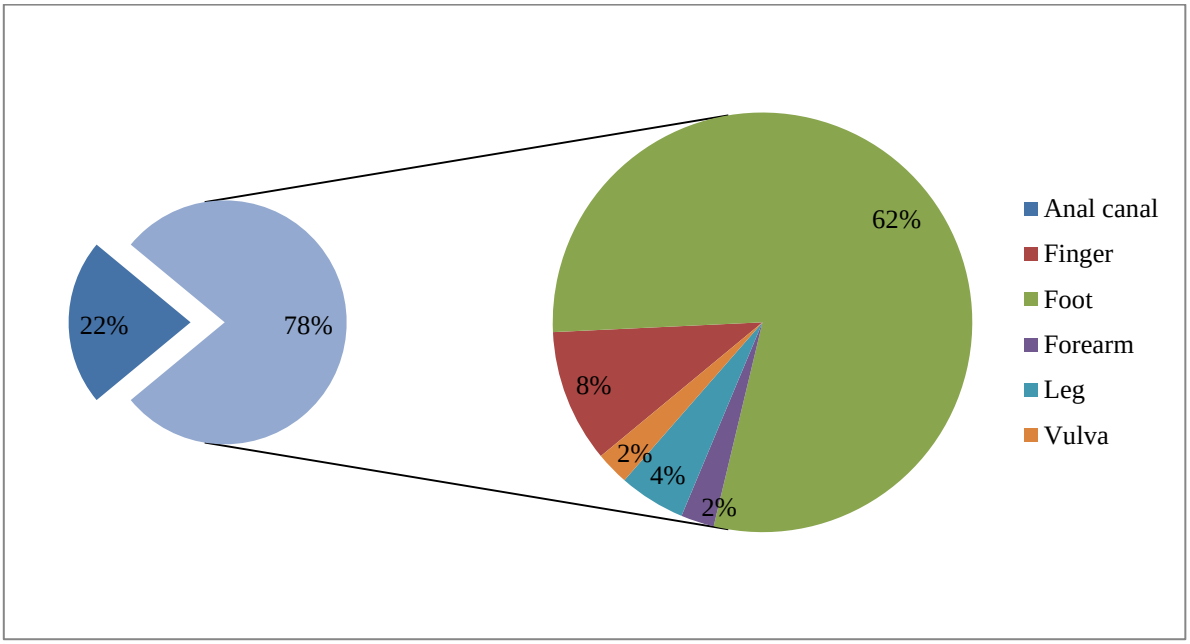


Figure 6. Distribution by clinical site in melanoma

Acral lentiginous and nodular subtypes of melanoma had equal prevalence (44%, n=22) in our study, with superficial spreading melanomas (4%, n=2) being the least common subtype. We did not find any case of lentigo maligna melanoma in our study. Acral lentiginous melanoma (56.4%, n=22) was noted to be the most common subtype of cutaneous melanoma while all cases (n=11) of anal melanomas were found to display a nodular invasive component. We also found one each of four other rare histological variants, namely pigment synthesising melanoma, rhabdoid melanoma, spitzoid melanoma and balloon cell melanoma. **(Fig. 7, Figs. 29-35)** In situ component of melanoma in the overlying epidermis was noted in all cutaneous melanomas. Of all cases of anal melanomas, four cases did not show identifiable epithelium in the biopsy and thereby the presence of in situ component could not be commented in those cases. The most common subtype of in situ component was also acral lentiginous (70%, n=35) followed by superficial spreading (22%, n=11) subtype. **(Fig. 57)**

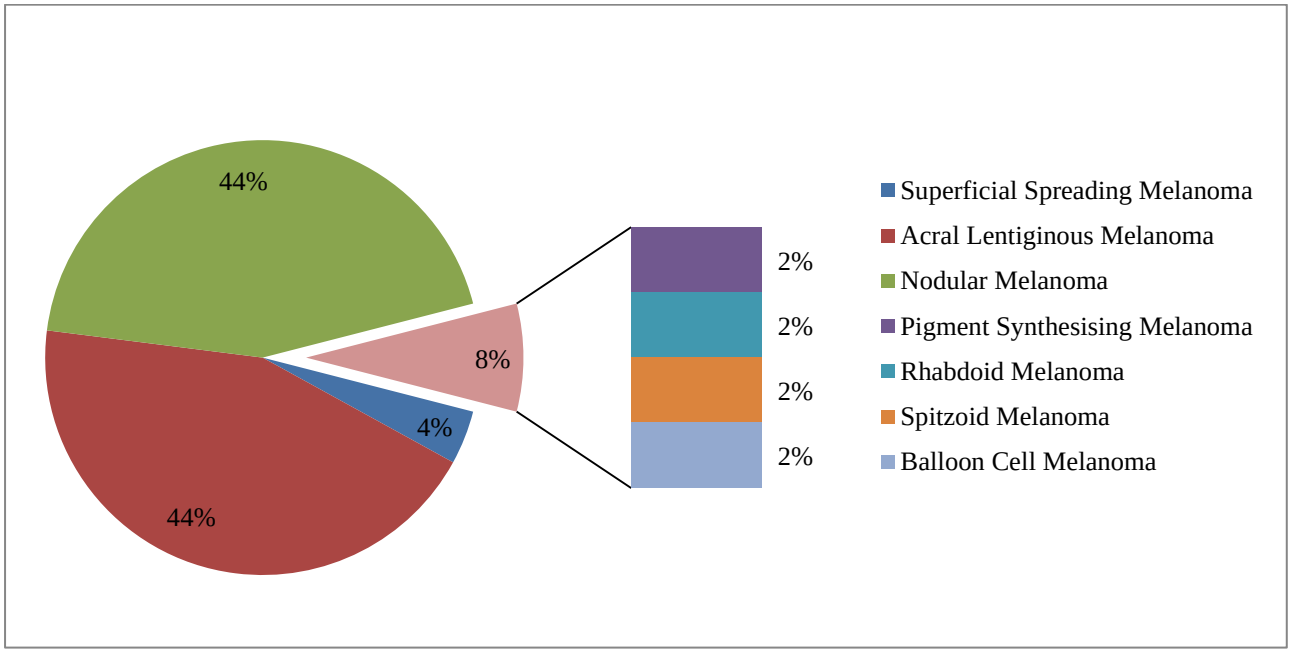


Figure 7. Histological subtypes of invasive melanoma

The maximum size of tumour at the time of presentation ranged from 0.5 cm to 10.0 cm (median: 2.9 cm). Cutaneous melanomas presented with tumours almost twice as large (median: 3.0 cm, 0.5-10.0 cm) as compared to anal melanomas (median: 1.5 cm, 1.0-6.0 cm). The maximum thickness of tumour as measured by Breslow's method ranged from 0.63 mm to 55.00 mm, with a median value of 6.84 mm. The median tumour thickness in cutaneous and anal melanomas were 6.55 mm (0.63-55.00 mm) and 7.80 mm (1.30-25.00 mm) respectively. **(Fig. 8)** Almost 96% (n=48) of all cases were tumours with Clark level of invasion 4 and 5. **(Fig. 58)** While majority (96%, n=48) of tumours showed vertical phase of growth, only 2 (4%) cases displayed a radial growth phase, the involved sites being vulva and foot.

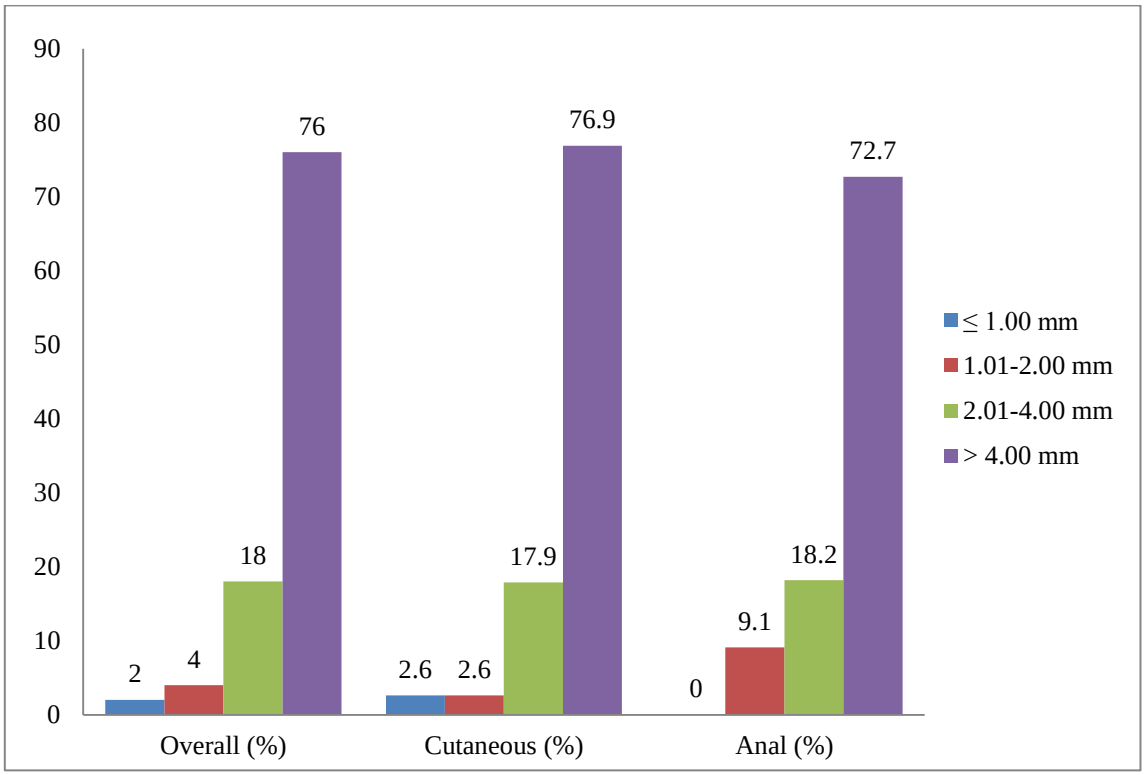


Figure 8. Categories of Breslow thickness in melanoma

Ulceration was present in 44 (88%) cases with all anal melanomas (n=11) displaying ulceration. **(Figs. 9,36)** Lymphovascular and perineural invasion were present in 70% (n=35) and 42% (n=21) cases respectively. **(Figs. 37-38, Figs. 59-60)** The median mitotic index of all cases of melanomas was found to be 12/mm^2 (range: 0-74/mm^2), with cutaneous and anal melanomas also having the same median value. **(Fig. 39)**

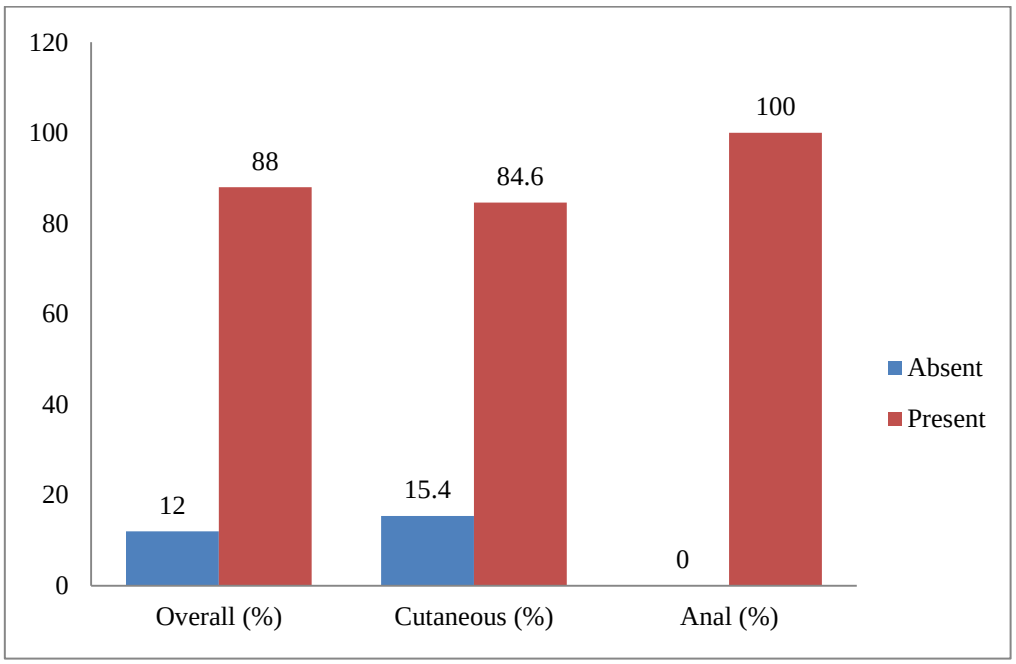


Figure 9. Ulceration in melanoma

Brisk response of tumour infiltrating lymphocytes was seen in 22% (n=11) while absent response was seen in only 6% (n=3) of all cases under study. None of the anal melanomas showed absent response. Non brisk response of tumour infiltrating lymphocytes was the most common (72%, n=36) response in our study. **(Figs. 41-42, Fig. 61)**

Regression was not identified in any anal melanomas under study. It was present in 20.5% (n=8) cases of cutaneous melanomas, of which 75% (n=6) and 25% (n=2) showed an extent of regression of 0-25% and 25-50% respectively. **(Figs. 10,40)**

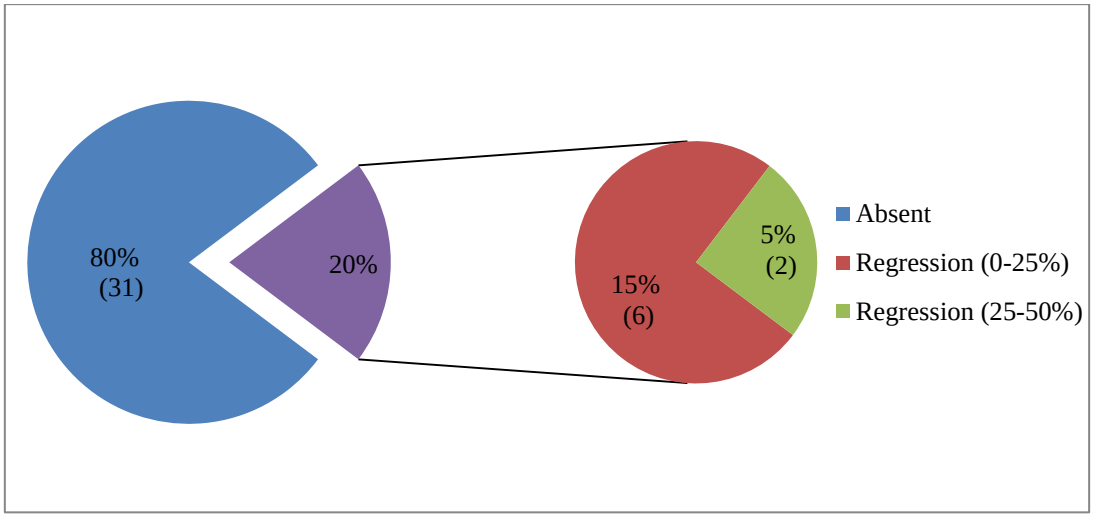


Figure 10. Extent of regression in cutaneous melanomas

Bone invasion was present only in 2 (14.3%) out of 14 cutaneous melanomas **(Fig. 44)** None of the cases showed evidence of sunlight induced damage assessed by solar elastosis. Majority of the tumours displayed cellular pigmentation (76%, n=38) of which 24 (63.2%) cases showed very high granular cytoplasmic pigmentation. **(Fig. 11, Table 11)** Tumour giant cells were present in 6(12%) cases under study, 5(83.3%) of which were cutaneous melanomas. **(Fig. 43)** Upward scatter (82%, n=41) and nest formation of the atypical melanocytes (80%, n=40), lateral circumscription of the overlying epidermis (continuous 86%, n=43) and epidermal hyperplasia (72%, n=36) were also noted. **(Figs. 45-46, Figs. 62-64, Tables 12-15)** Epithelioid and spindled cell morphology were found in 32 (64%) and 18 (36%) cases respectively in our study. **(Figs. 47-48, Fig. 66, Table 16)** Many tumours displayed large size of cell, nucleus and nucleolus (88%, n=44) **(Figs. 49-50, Fig.65)**

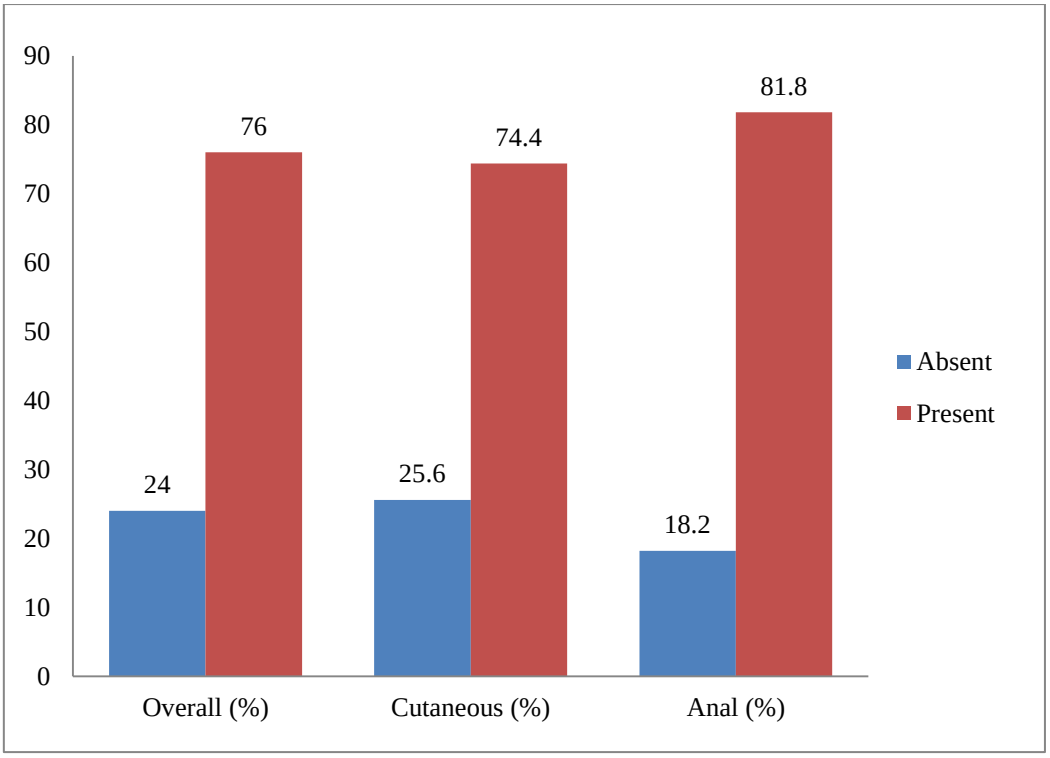


Figure 11. Cellular pigmentation in melanoma

Satellite lesions/ in transit metastases were found clinically in 14 (35.9%) patients with cutaneous melanomas, 9 of which 9 confirmed by histopathological examination. **(Figs. 51-52)** One case showed satellite lesion detectable only by microscopy while the patient did not have any clinically identifiable lesion. All anal melanomas did not have any satellite lesions. Only exception was one case wherein there was a multifocal synchronous tumour.

Immunohistochemical studies were carried out to confirm the diagnosis in 43 (86%) cases. The most common immunohistochemical markers used were HMB-45, S100 and Melan A. **(Fig. 55)** Apart from these markers, CD117 was performed in 2 cases (vulva and toe), which turned out to be positive.

Lymph node dissection was performed in 14 (28%) cases which included 11 (78.6%) cutaneous and 3 (21.4%) anal melanomas. All the surgical specimens were therapeutic lymphadenectomy. Sentinel lymph node biopsy was not performed in any case. In keeping with the most common site of lower limb involvement in cutaneous melanomas, 9 (81.8%) patients underwent block dissection of inguinal lymph nodes. **(Table 17)** The maximum size of lymph node ranged from 0.5 cm to 10.0 cm with a median value of 3.3 cm. The median value of maximum lymph node size in cutaneous and anal melanomas were 4.0 cm (1.0-10.0 cm) and 1.2 cm (0.5-3.0 cm) respectively. Of the 14 patients who underwent therapeutic lymphadenectomy, 11 (78.6%) cases showed metastatic tumour deposits in the nodes, 6 of which displayed extracapsular invasion. **(Fig. 12, Figs. 53-54)**

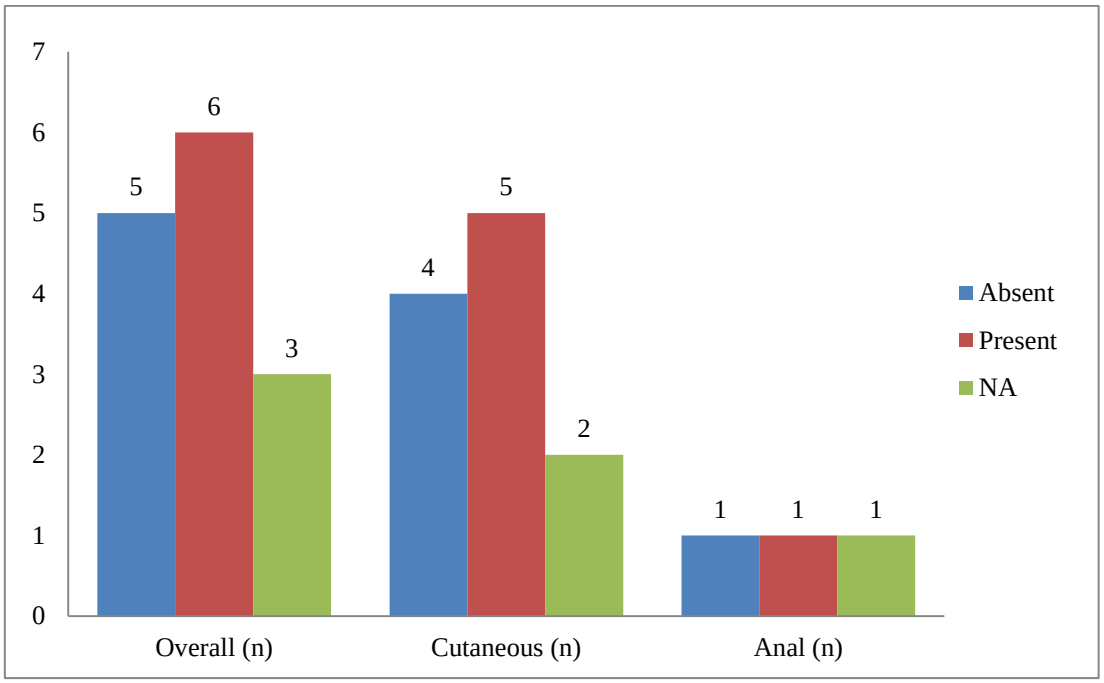


Figure 12. Extracapsular extension of tumour in lymph nodes (NA-Not Applicable)

Fine needle aspiration of clinically suspicious lymph nodes followed by cytological examination were performed in 8 cases of cutaneous melanomas, of which 87.5% (n=7) turned out to be positive.

Serum LDH levels were available only in 9 (18%) cases. The normal limits of serum LDH in our Biochemistry laboratory ranged from 225-460 U/L. The measurements of serum LDH levels ranged from 337.0 to 2383.8 U/L, with a median value of 449 U/L. **(Fig. 13)**

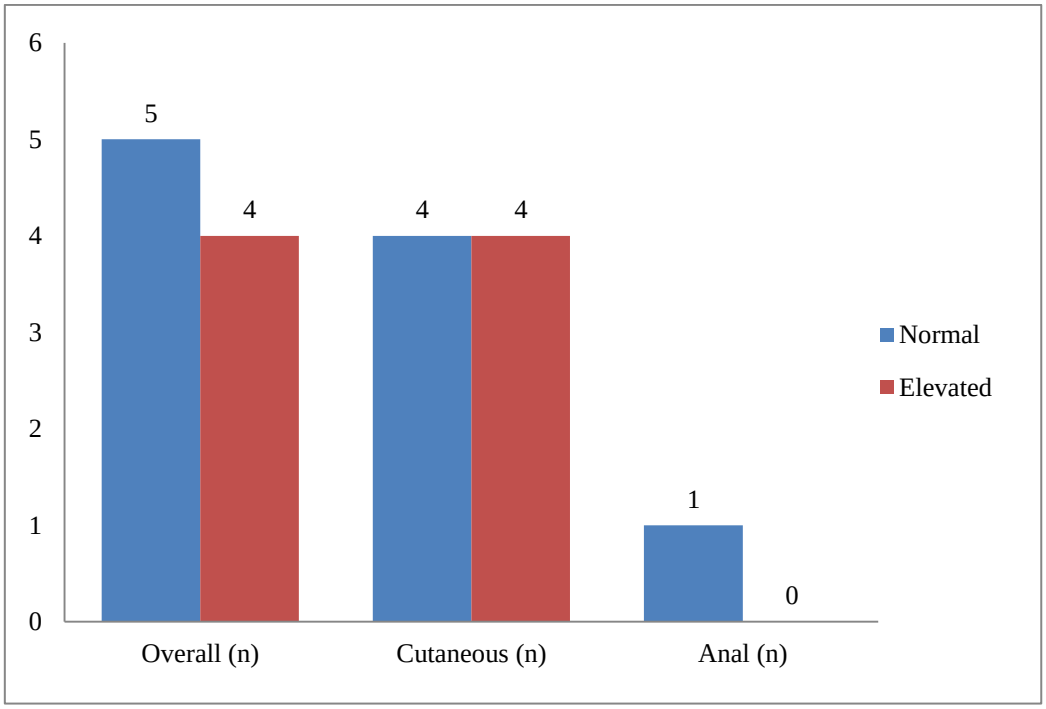


Figure 13. Degree of serum LDH levels in melanoma (LDH-Lactate Dehydrogenase)

Adjuvant therapy was provided in 12 (24%) cases under study. **(Fig. 67)** Nine (75%) patients were offered chemotherapy. The most common chemotherapeutic drugs given were Temozolamide, Paclitaxel and Dacarbazine. Two (16.7%) patients were

provided with interferon therapy while one (8.3%) patient with cutaneous melanoma was offered both chemotherapy and interferon therapy.

Ten (20%) patients were completely lost to follow-up. The median follow-up period was 14.5 months (2-50 months). We were able to contact only 25 (50%) patients to assess the survival status. Only 28% (n=7) patients were alive at the end of the study period (as on August 31, 2017). **(Fig. 14)**

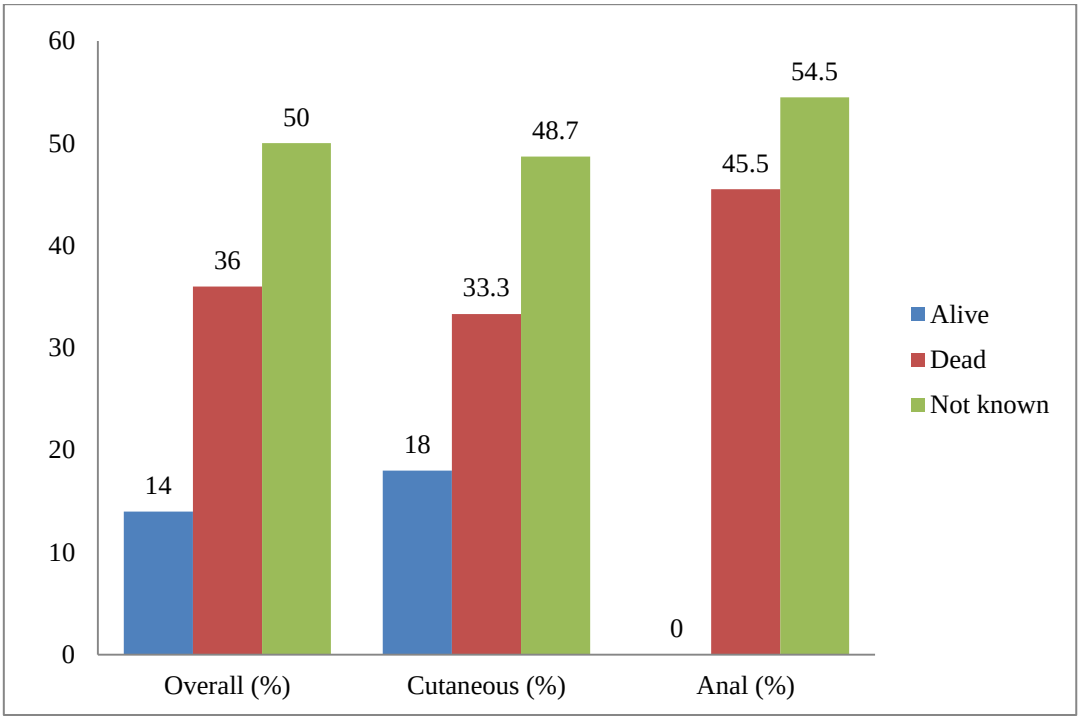


Figure 14. Survival status of patients with melanoma

Only 50% (n=25) patients underwent a definitive surgical procedure (excision/ resection), and therefore information about recurrence was collected only from these cases. Recurrence was present in 20% (n=5) cases, of which 3 were anal melanomas.

Distant metastases were present in 46% (n=23) cases. **(Fig. 68)** While majority of the patients with cutaneous melanomas (85.7%, n=12) developed distant metastases during the follow-up period, a large proportion of patients with anal melanomas (77.8%, n=7) had metastases on presentation. **(Fig. 15)** The most common organs involved by metastases were liver (69.6%) and lung (43.5%). One patient with cutaneous melanoma had metastasis to the brain. **(Fig. 16-17, Table 18)** Twelve (52.2%) patients had single organ metastases, while patients who had anal melanomas displayed propensity to develop metastases in multiple sites. **(Fig. 69)**

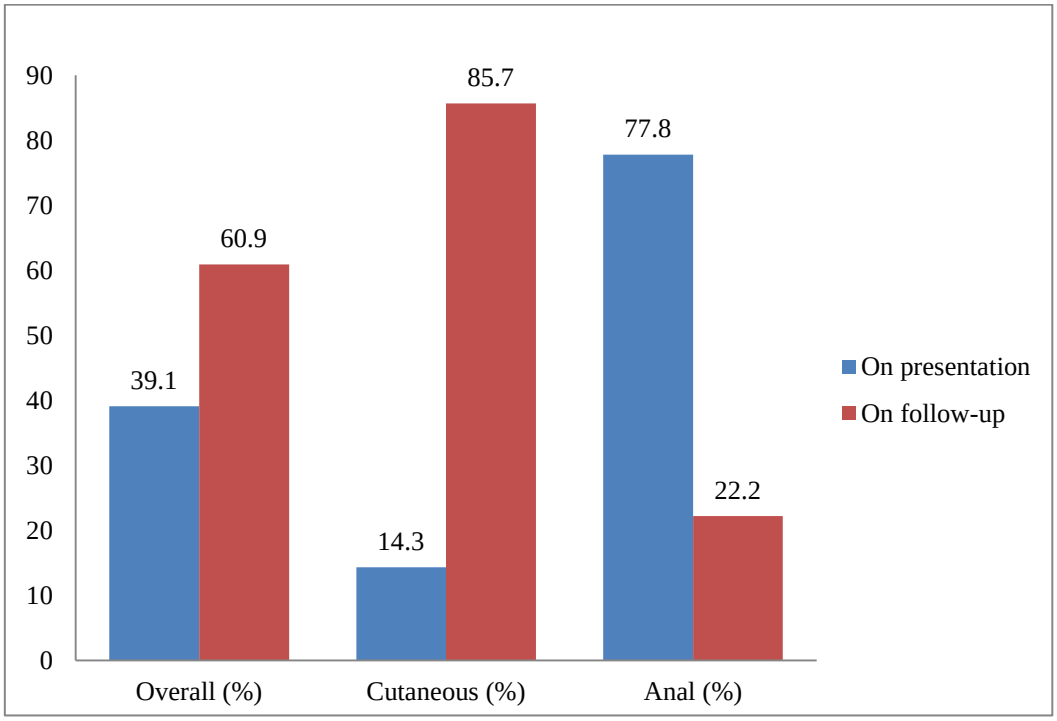


Figure 15 . Time of onset of metastases in melanoma

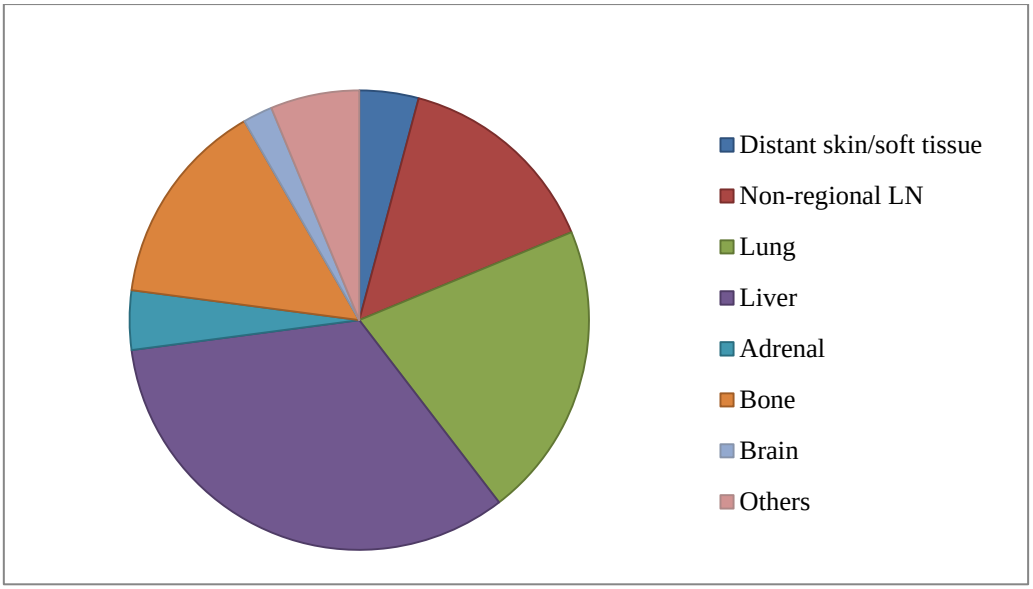


Figure 16. Distribution by site of metastases in melanoma (LN-Lymph Node)

Thirty eight (76%) patients had Breslow tumour thickness of > 4.00 mm. As expected, majority of tumours (74%, n=37) were in the T4 category followed by 20% (n=10) in T3 category. One cutaneous melanoma, arising from the vulvar region, had a tumour stage of T1 (pT1a). As anal melanomas did not have any standard pathological staging criteria, they were classified according to the AJCC (2010) pathological classification in this study. Majority of the anal melanomas (63.6%, n=7) were of T4 stage (pT4b). **(Fig. 18, Table 19)**

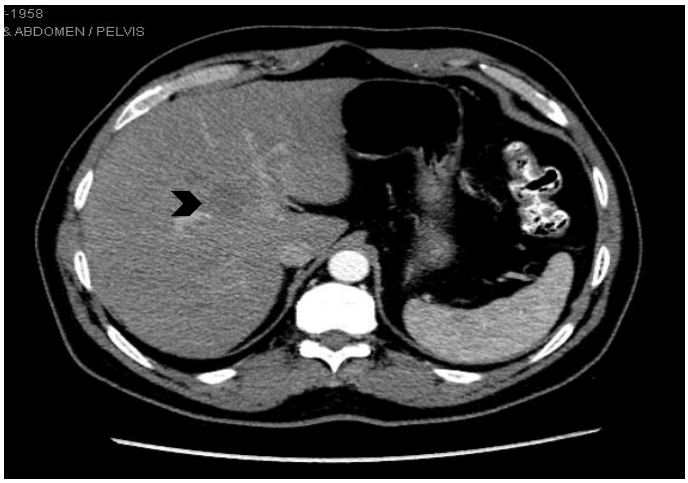


Figure 17. CT scan showing metastatic lesions in liver (indicated by arrowhead)

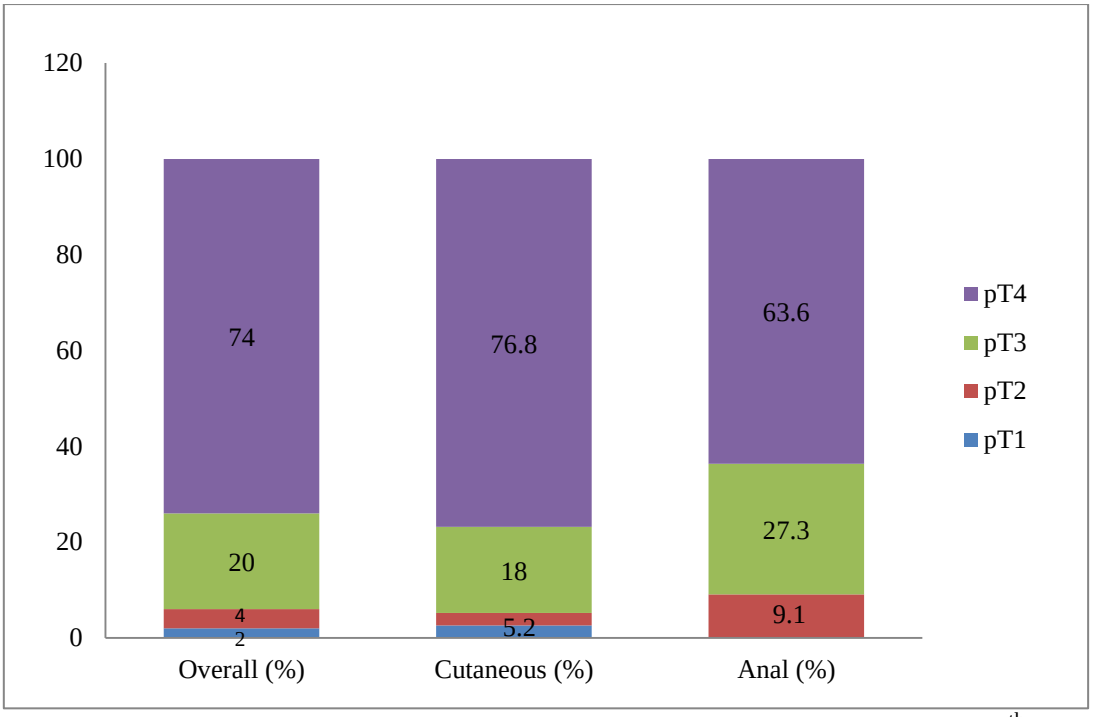


Figure 18. Distribution by pathological T (pT) stage in melanoma – AJCC 7th ed. classification

In cases where lymph node staging could be assessed, 87% (n=20) cases were in N2 and N3 categories. **(Fig. 19, Table 20)** In a large proportion of patients, the diagnosis of metastases was made only clinically or by radiography. All cases of anal melanomas with clinically detectable metastases (81.8%, n=9) were in the cM1c category. **(Fig. 20, Table 21)**

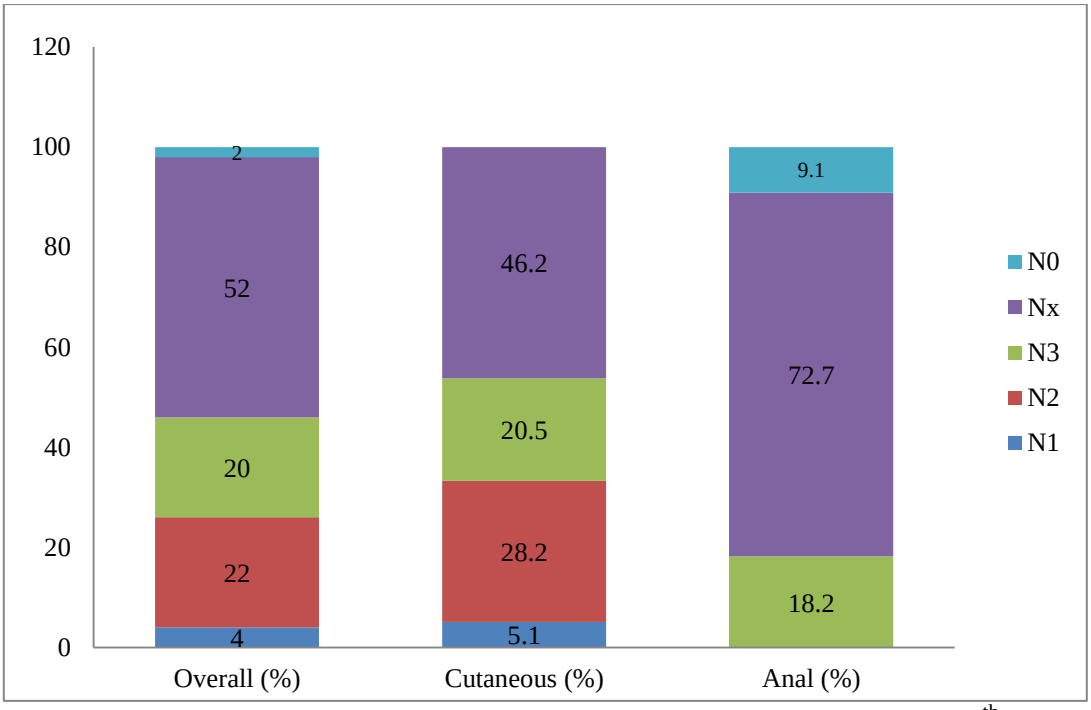


Figure 19. Distribution by pathological N (pN) stage in melanoma – AJCC 7th edition classification

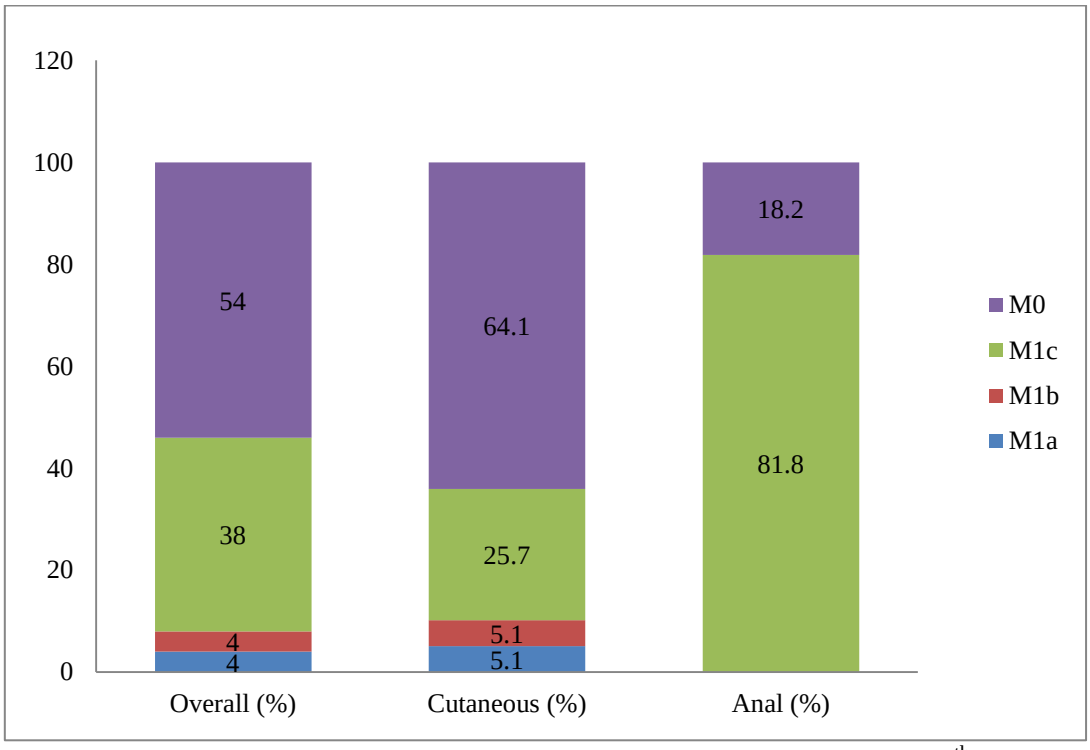


Figure 20. Distribution by clinical M (cM) stage in melanoma – AJCC 7th edition classification

In our study, we noted that melanomas occurring on fingers (n=4) were seen only in males. Majority of melanomas of foot (83.9%, n=26) displayed a maximum tumour size of ≤ 4 cm, while 66.7% (n=2) of melanomas occurring on legs and forearms were of size > 4 cm. All the cases of melanomas of leg/forearm region (n=3) exhibited a nodular subtype and cytoplasmic pigmentation, while only those melanomas occurring on foot (n=8) developed regression. Recurrence was seen in only 2 cutaneous melanomas, both of which were in the foot. **(Table 22)**

Anal melanomas were found to display a higher rate of recurrence (p=0.038) and metastases (p=0.014) as compared to cutaneous melanomas. Anal melanomas displayed a higher rate of metastases on presentation, while cutaneous melanomas were associated with development of metastases on follow-up (p=0.007). Nodular melanomas also displayed a higher rate of metastases on presentation, while acral lentiginous subtype was associated with development of metastases at a later stage in the course of the disease (p=0.029). While cutaneous melanomas were associated with higher rate of single organ metastases, anal melanomas displayed multiple organ metastases (p=0.036). Anal melanomas were also found to be thicker tumours with nodular subtype and exhibiting increased depth of invasion, Clark Level V (p=0.016) as compared to cutaneous melanomas. In our study, males were noted to present with a smaller tumour size of ≤ 4 cm at presentation (p=0.026) and ulceration (p=0.018) as compared to females. Acral lentiginous melanomas were associated with elevated levels of serum LDH as compared to nodular melanomas which predominantly showed serum levels within normal range (p=0.048). **(Table 4, Tables 23-27)**

Table 4. Univariate analysis of clinicopathological parameters with clinical site

Characteristics	Clinical Site		p value
	Cutaneous (n=39)	**Anal (n=11)**	
Age (in years)			0.082
≤ 50	16 (41)	8 (72.7)	
51-60	12 (30.8)	3 (27.3)	
> 60	11 (28.2)	0	
Gender			0.724
Male	24 (61.5)	8 (72.7)	
Female	15 (38.5)	3 (27.3)	
Max Tumour Size			0.271
≤ **2 cm**	15 (38.5)	7 (63.6)	
2-4 cm	16 (41)	2 (18.2)	
> 4 cm	8 (20.5)	2 (18.2)	
Invasive Subtype			**< 0.001**
SSM	2 (5.1)	0	
ALM	22 (56.4)	0	
Nodular	11 (28.2)	11 (100)	
Others	4 (10.3)	0	
Breslow Thickness (in mm)			> 0.99
≤ **4.00**	9 (23.1)	3 (27.3)	
> 4.00	30 (76.9)	8 (72.7)	
Clark Level*			**0.016**
4	19 (51.4)	1 (9.1)	
5	18 (48.6)	10 (90.9)	
Growth Phase			> 0.99
Radial	2 (5.1)	0	
Vertical	37 (94.9)	11 (100)	
Ulceration			0.317
Absent	6 (15.4)	0	
Present	33 (84.6)	11 (100)	
Lymphovascular Invasion			0.713
Absent	11 (28.2)	4 (36.4)	
Present	28 (71.8)	7 (63.6)	
Perineural Invasion			0.741
Absent	22 (56.4)	7 (63.6)	
Present	17 (43.6)	4 (36.4)	
Tumour Infiltrating Lymphocytes			> 0.99
Absent	3 (7.7)	0	
Non brisk	27 (69.2)	9 (81.8)	
Brisk	9 (23.1)	2 (18.2)	
Regression			0.174
Absent	31 (79.5)	11 (100)	
Present	8 (20.5)	0	
Mitotic Index (per mm^2)			> 0.99
≤ 6	11 (28.2)	3 (27.3)	
6-12	9 (23.1)	3 (27.3)	
> 12	19 (48.7)	5 (45.4)	
Cellular Pigmentation			> 0.99
Absent	10 (25.6)	2 (18.2)	
Present	29 (74.4)	9 (81.8)	
Upward Scatter*			0.111
Absent	4 (10.3)	3 (33.3)	
Present	35 (89.7)	6 (66.7)	
Nest Formation*			0.159
Absent	5 (12.8)	3 (33.3)	
Present	34 (87.2)	6 (66.7)	
Lateral Circumscription*			> 0.99

Abrupt	2 (5.3)	0	
Continuous	36 (94.7)	7 (100)	
Cell Size			0.111
Medium	3 (7.7)	3 (27.3)	
Large	36 (92.3)	8 (72.7)	
Cell Shape			0.287
Epithelioid	23 (59)	9 (81.8)	
Spindled	16 (41)	2 (18.2)	
Clinical Satellite/In transit metastases			**0.022**
Absent	25 (64.1)	11 (100)	
Present	14 (35.9)	0	
Max Lymph Node Size			0.636
≤ 3 cm	3 (33.3)	2 (100)	
3-6 cm	4 (44.4)	0	
> 6 cm	2 (22.2)	0	
Extranodal Tumour Extension			> 0.99
Absent	4 (44.4)	1 (50)	
Present	5 (55.6)	1 (50)	
pT Stage*			0.424
T3	7 (18.9)	3 (30)	
T4	30 (81.1)	7 (70)	
pN Stage*			0.214
N2	11 (57.9)	0	
N3	8 (42.1)	2 (100)	
pN Stage (8^{th} Ed)*			0.718
N1	13 (61.9)	0	
N3	8 (38.1)	2 (100)	
cM Stage*			**0.004**
M1c	10 (28.6)	9 (81.8)	
M0	25 (71.4)	2 (18.2)	
No of Sites with Distant Metastases			**0.036**
Single	10 (71.4)	2 (22.2)	
Multiple	4 (28.6)	7 (77.8)	
Adjuvant Treatment			0.105
Absent	32 (82.1)	6 (54.5)	
Present	7 (17.9)	5 (45.5)	
Serum LDH Levels			> 0.99
Normal	4 (50)	1 (100)	
Elevated	4 (50)	0	

* All cases not included
Abbreviations: SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

The overall survival (OS) rate at the end of 1 year, 2 years and 3 years were 77.8%, 22.2% and 5.6% respectively. The median overall survival period was 16 months (95% CI: 14-35 months). Lesser OS with shorter median time for occurrence of death was associated with medium cell size (p=0.005) and involved peripheral margin of invasive component (p=0.04). **(Figs. 21-22, Table 28)**

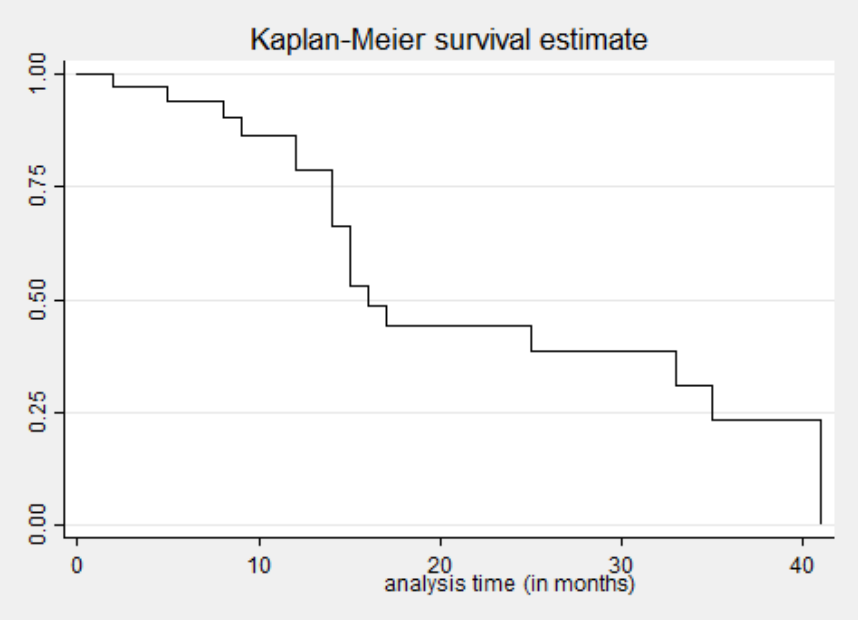


Figure 21. Overall survival (OS) in patients with melanoma

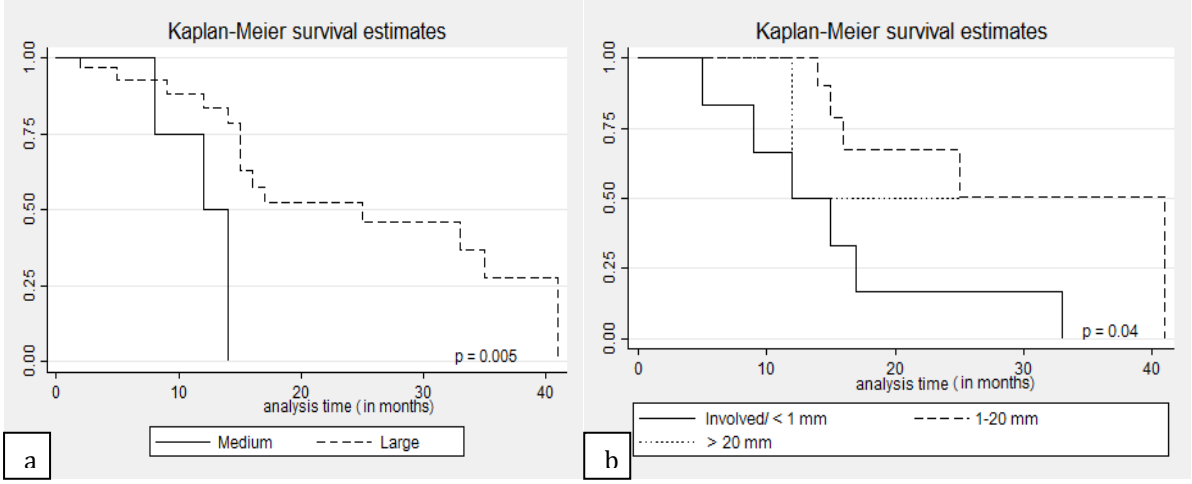


Figure 22. Overall survival (OS) in melanoma patients with respect to cell size(a) and invasive peripheral margin(b) ($p < 0.05$ significant)

We found that the distant metastases free survival (DMFS) rate at the end of 1 year, 2 years and 3 years were 64.3%, 28.6% and 14.3% respectively. The median time period for metastases to occur from the time of diagnosis was 25 months (95% CI: 8-36 months). The presence of clinically detectable satellite lesions/ in transit metastases

was significantly associated with lesser distant metastases free survival (p=0.006).

(Figs. 23-24, Table 29)

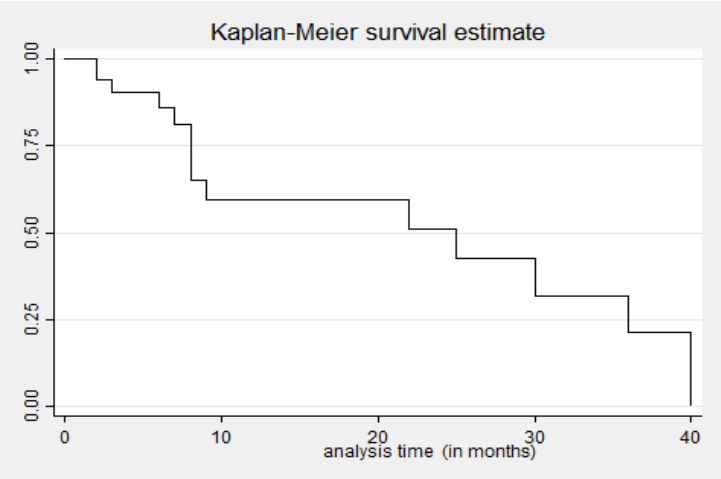


Figure 23. Distant metastases free survival (DMFS) in patients with melanoma

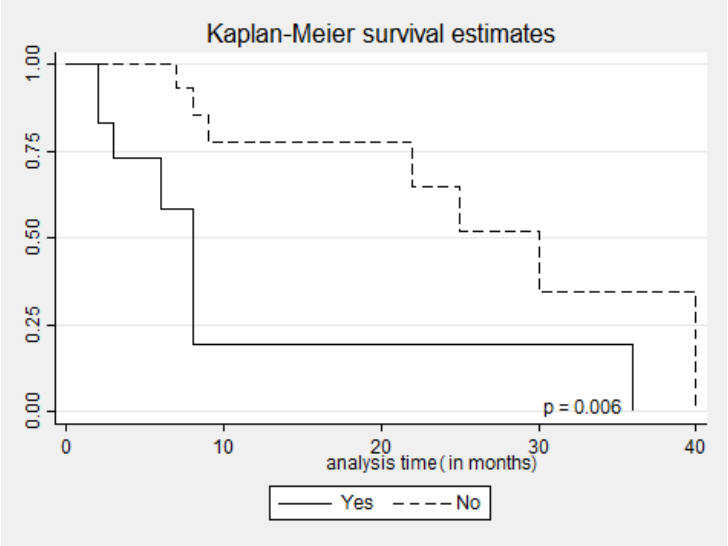


Figure 24. Distant metastases free survival (DMFS) in melanoma patients with respect to clinical satellite/ in transit metastases ($p < 0.05$ significant)

Recurrence was identified in only 5 cases. We found that 40% patients were free of recurrence at the end of one year. The mean time period for patients to develop recurrence was found to be 29.8 months (95% CI: 22.5-37.1 months). Factors associated with adverse outcome included female gender, anal melanomas with nodular subtype, higher Clark level, amelanotic tumours, epithelioid cell morphology,

involved margins, satellitosis/in transit metastases and presence of extranodal tumour extension, though the values were not statistically significant. **(Fig. 25, Figs. 78-79, Table 30)**

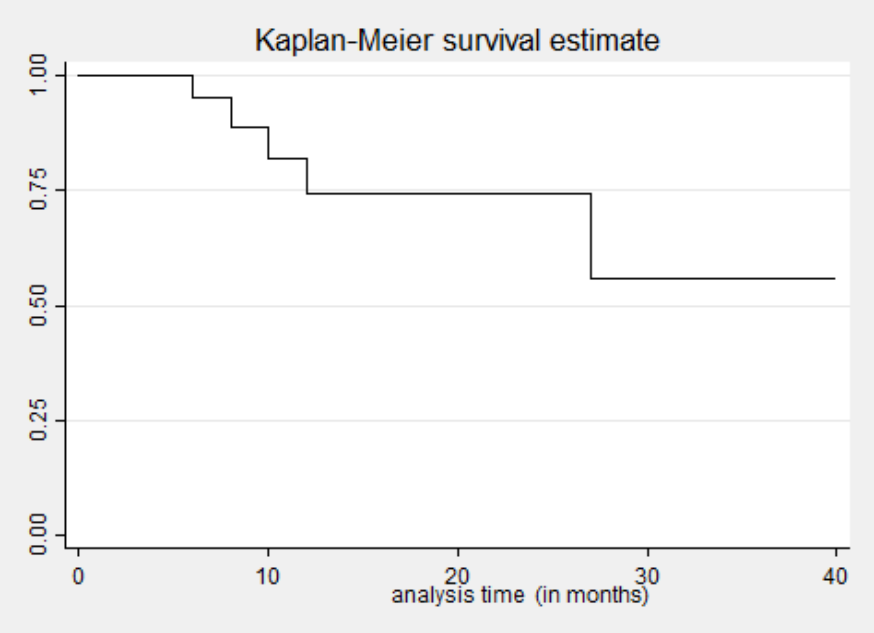


Figure 25. Recurrence free survival (RFS) in patients with melanoma

BRAF^{V600E} mutation analysis was performed in all the cases in our study. Forty five of fifty patients (90%) had amplifiable DNA in the tissue for the sequencing to be undertaken, while the remaining 5 (10%), including 4 cases of cutaneous and 1 anal melanoma displayed no amplification as detected by agarose gel electrophoresis, with controls being satisfactory. **(Fig. 27)** All the cases in which sequencing was performed (n=45), displayed absence of *BRAF*^{V600E} mutation. **(Fig. 26, Fig. 28, Figs. 80-81)** Based on the Bayesian methods, the lower and upper limits of prevalence in our population for *BRAF*^{V600E} mutation would be 0.2% and 8.8% respectively with a

median value of 2.7%. Thus, there is a possibility that on an average, the estimate of prevalence of *BRAF*^{V600E} mutation in our population would be 2.7%.

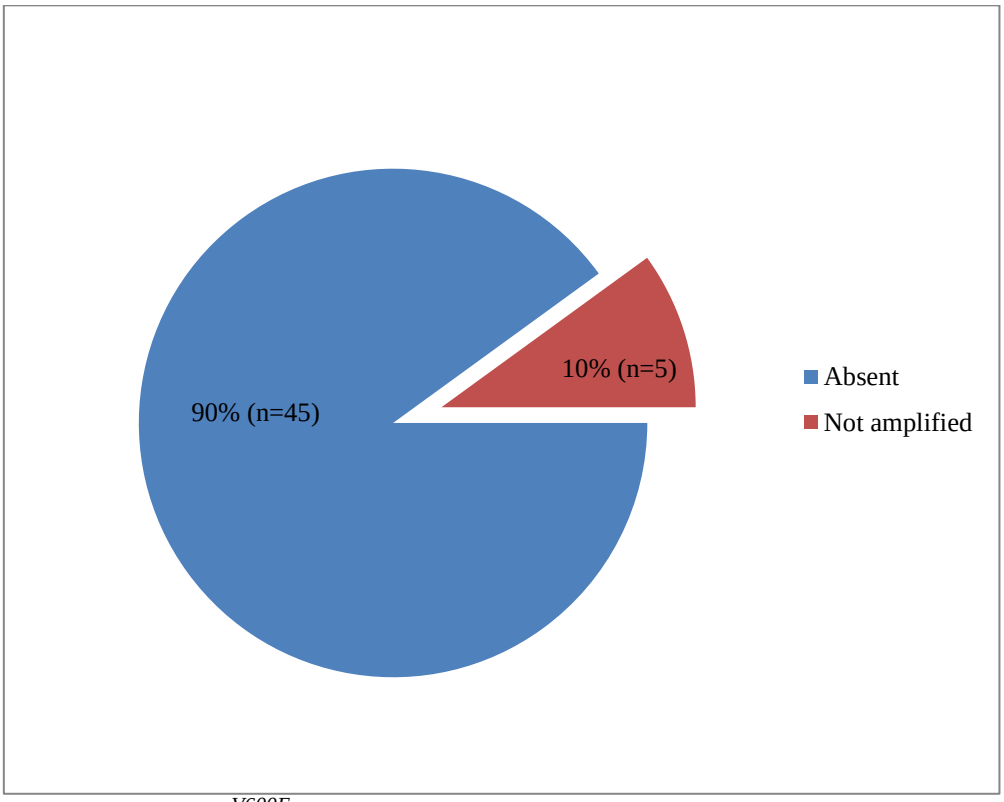


Figure 26. *BRAF*^{V600E} mutation status in melanoma

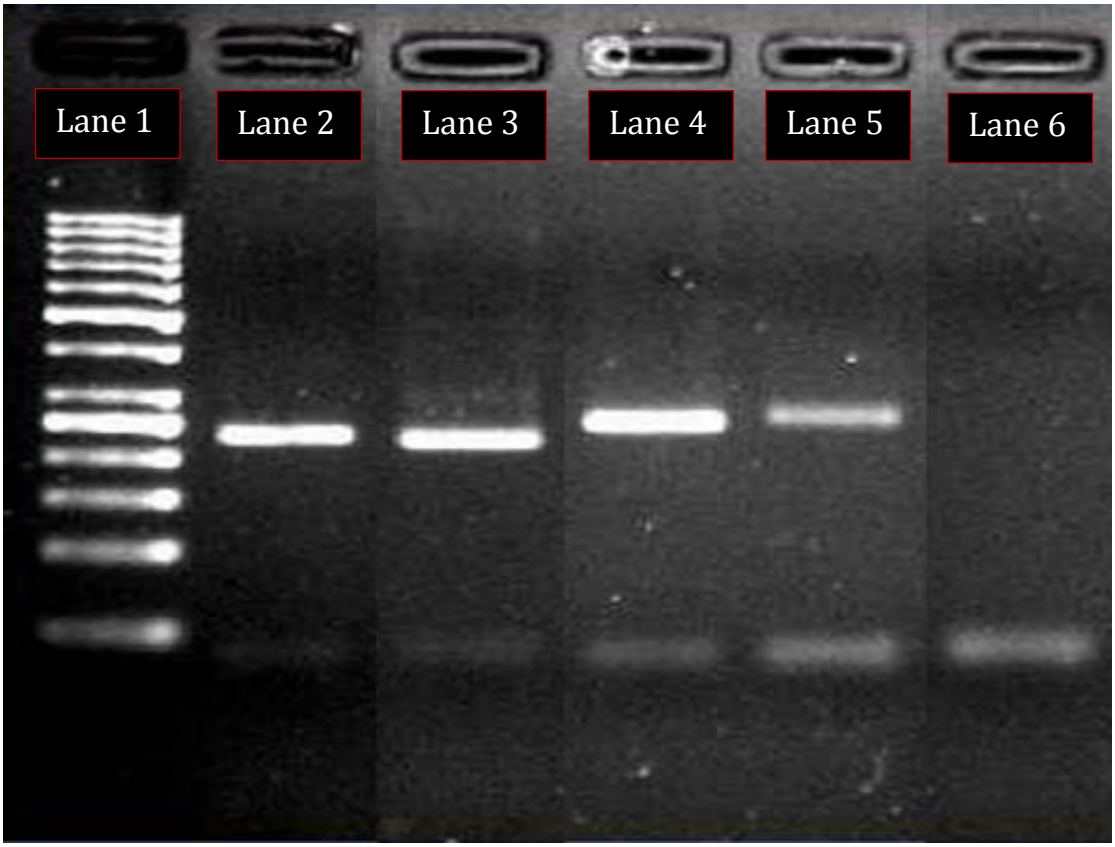


Figure 27. Gel electrophoresis – Positive at 230 bp for *BRAF*^{V600E} (Lanes 2-4); DNA Ladder (Lane 1), Positive control (Lane 5) and Negative control (Lane 6)

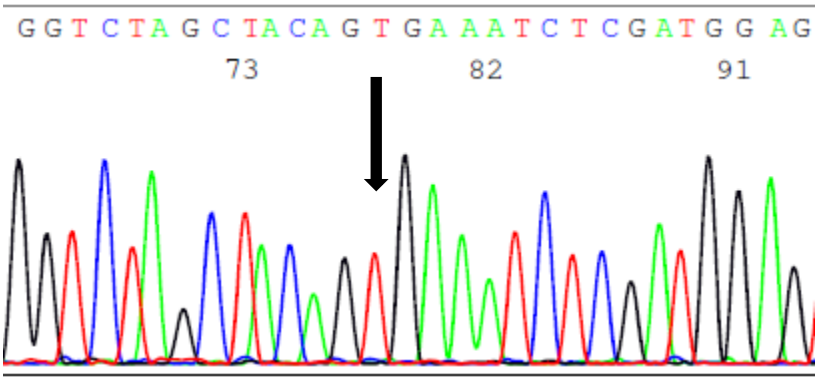


Figure 28. Sequencing for *BRAF*^{V600E} mutation, depicting absence of mutation - *BRAF wild type* (indicated by arrow)

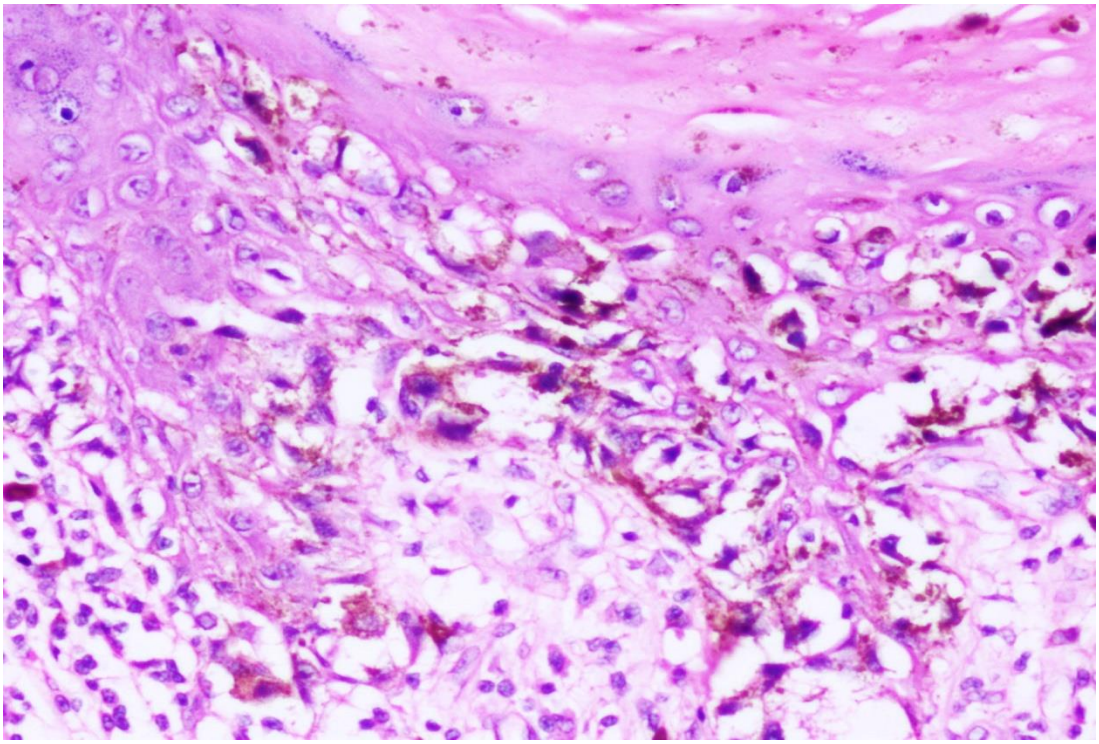


Figure 29. Superficial spreading melanoma (H&E, x200)

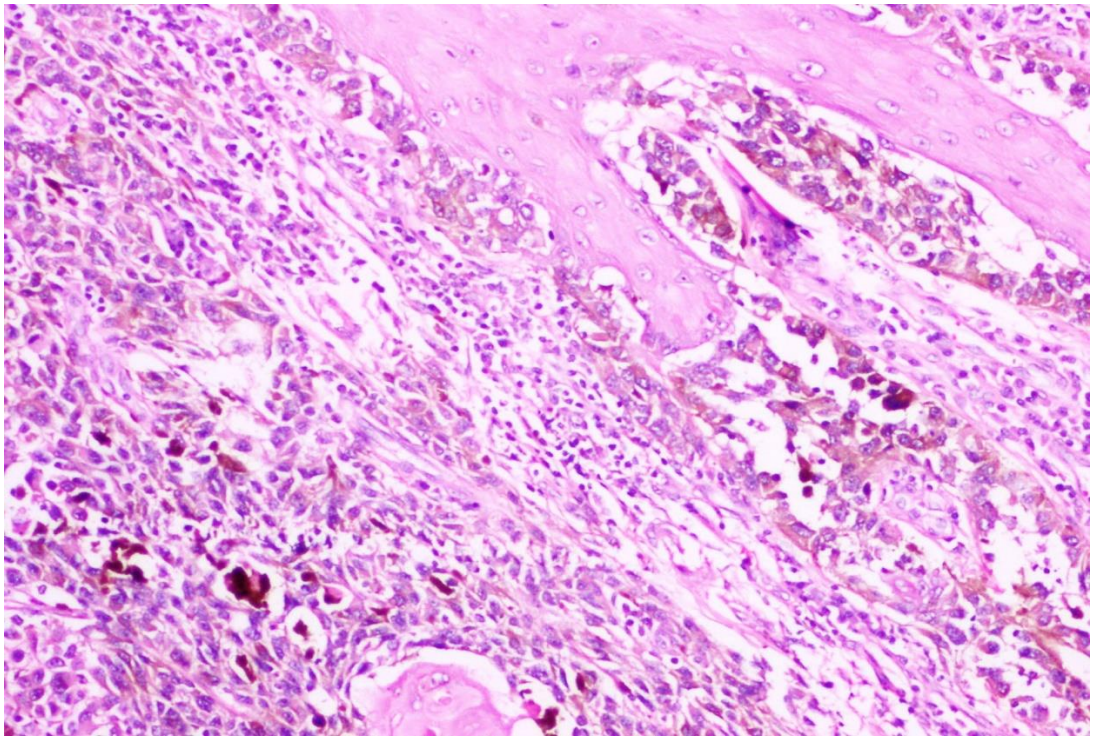


Figure 30. Acral lentiginous melanoma (H&E, x100)

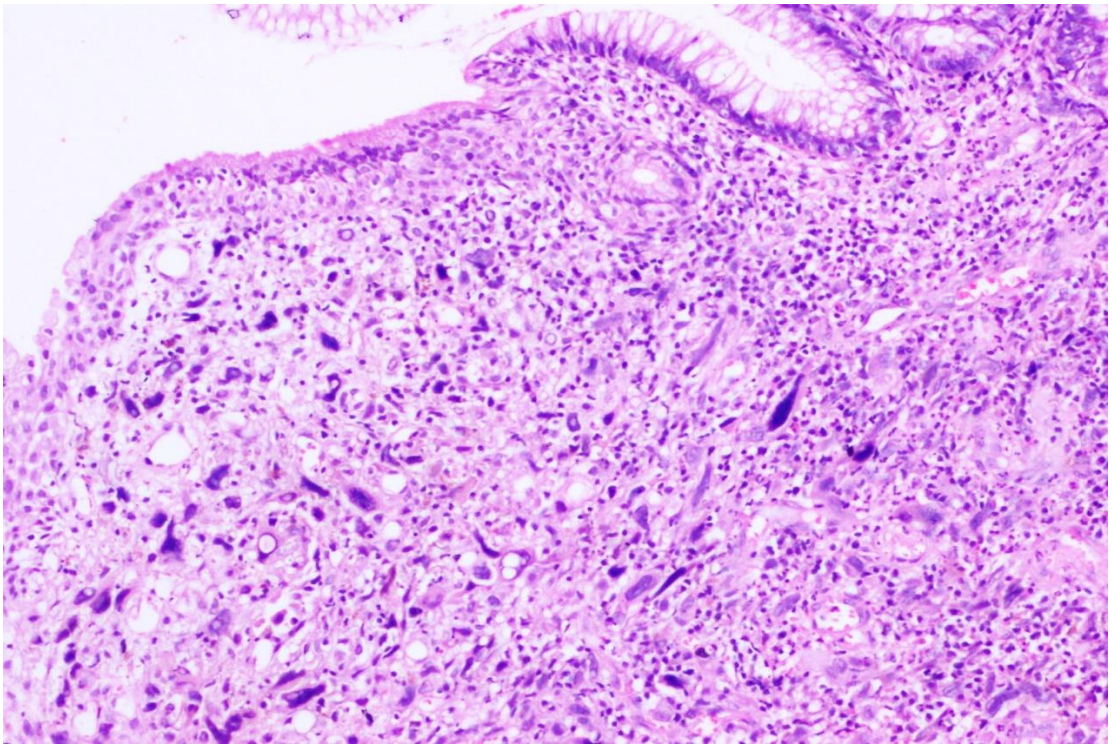


Figure 31. Nodular melanoma (H&E, x100)

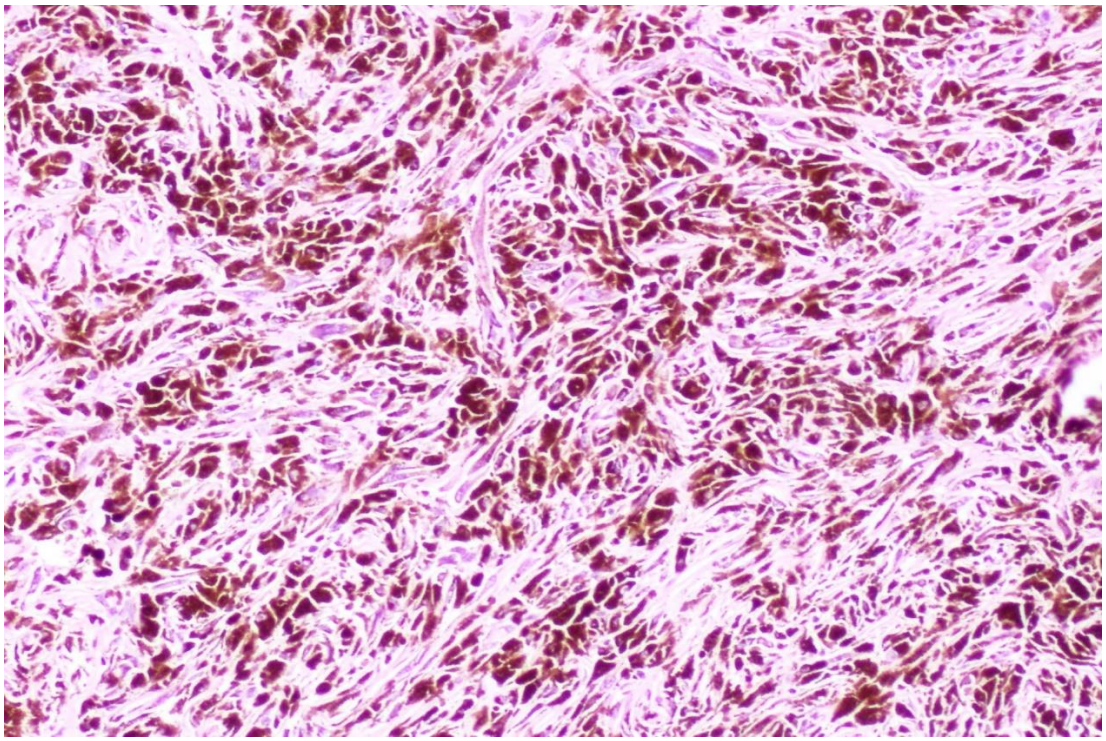


Figure 32. Pigment synthesising melanoma (H&E, x100)

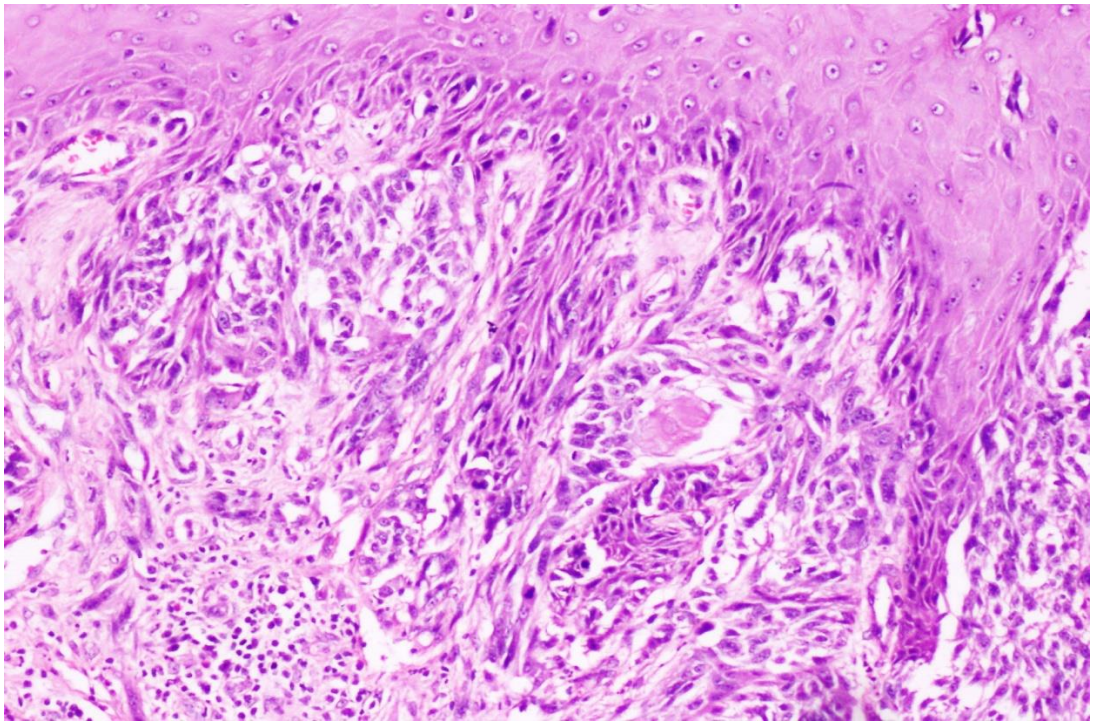


Figure 33. Spitzoid melanoma (H&E, x100)

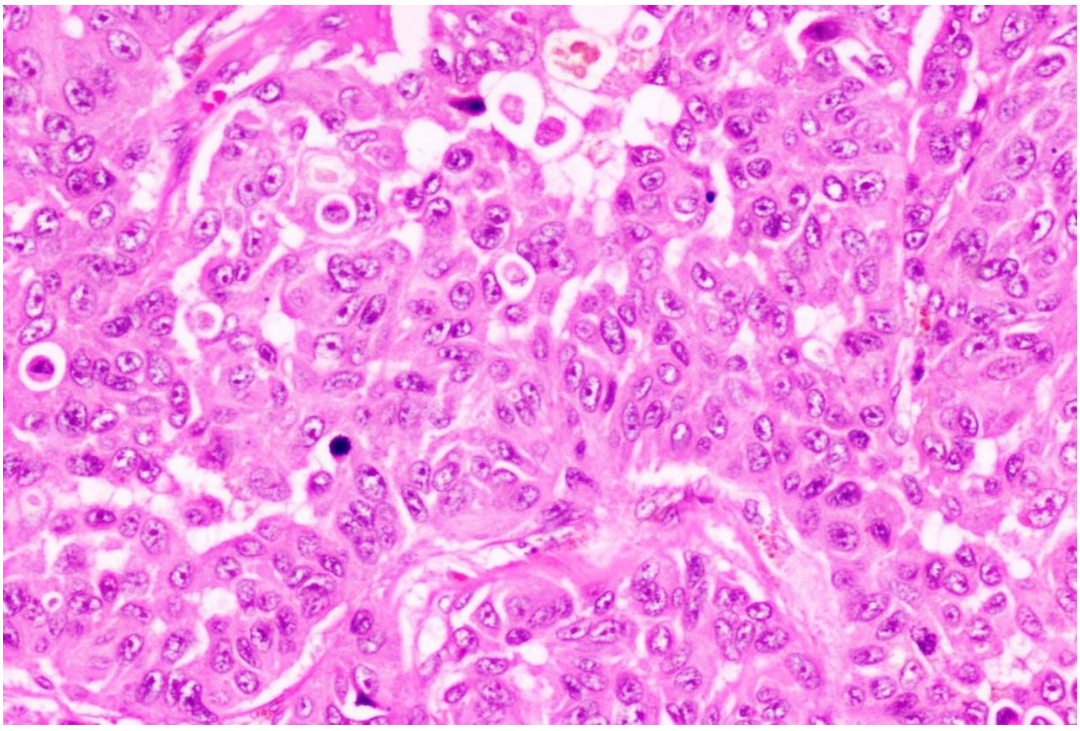


Figure 34. Rhabdoid melanoma (H&E, x200)

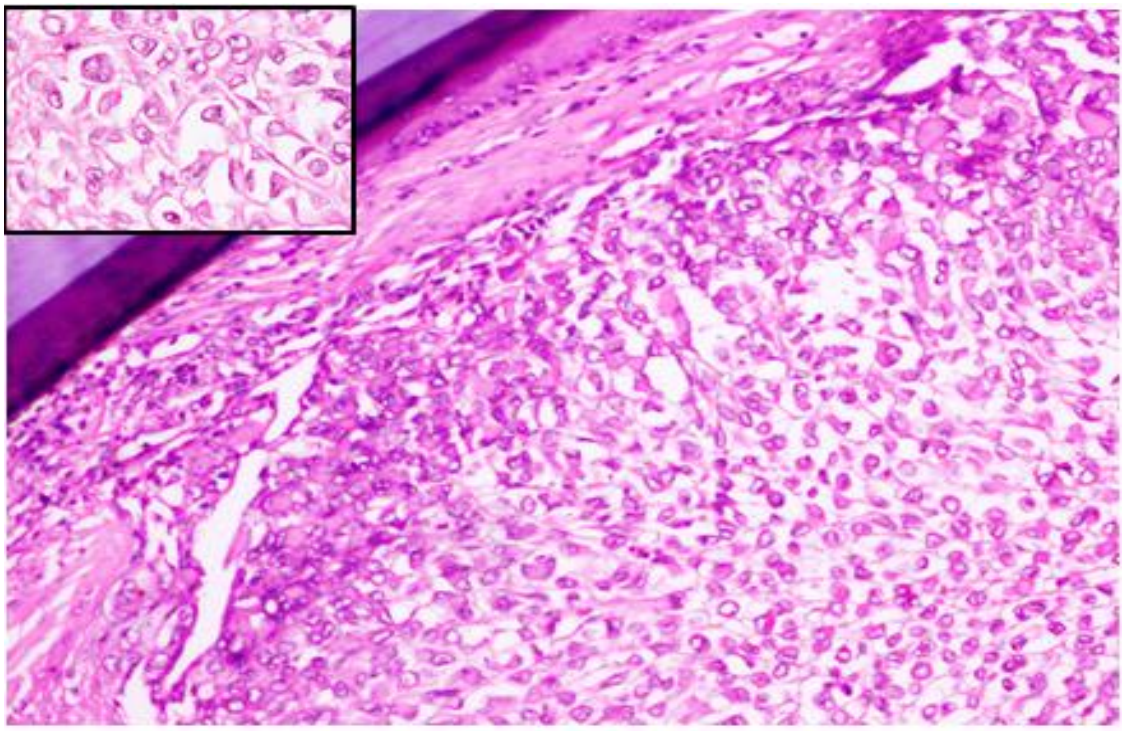


Figure 35. Balloon cell melanoma (H&E, x100); inset (x200)

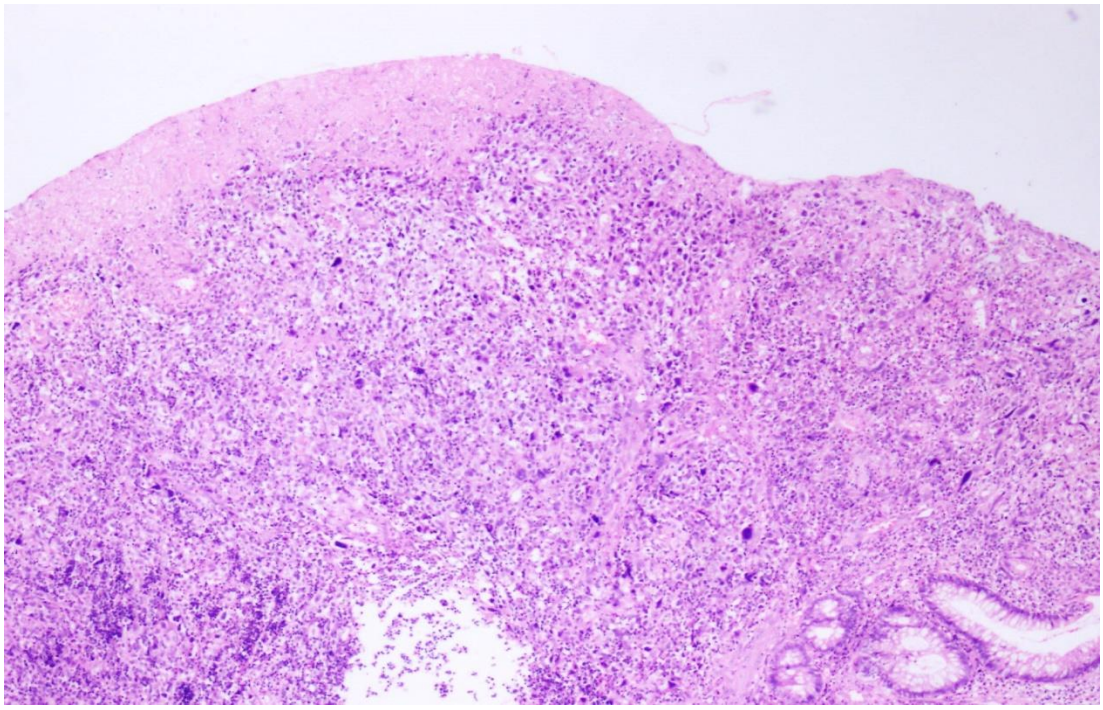


Figure 36. Anal melanoma with ulceration (H&E, x40)

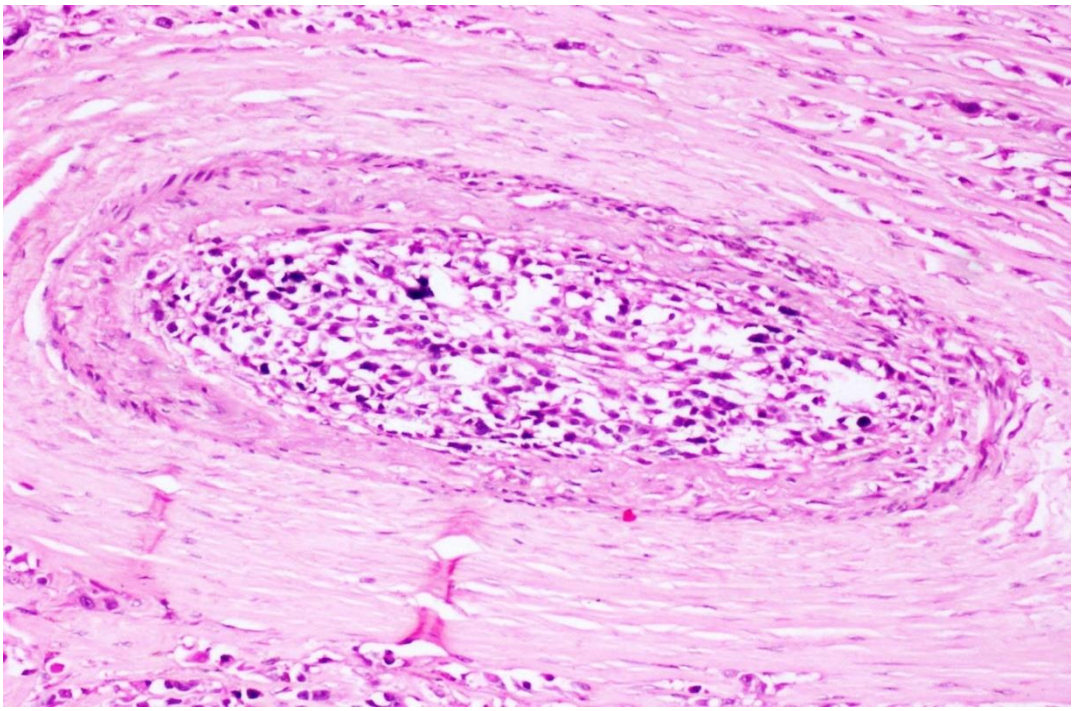


Figure 37. Lymphovascular invasion in melanoma (H&E, x100)

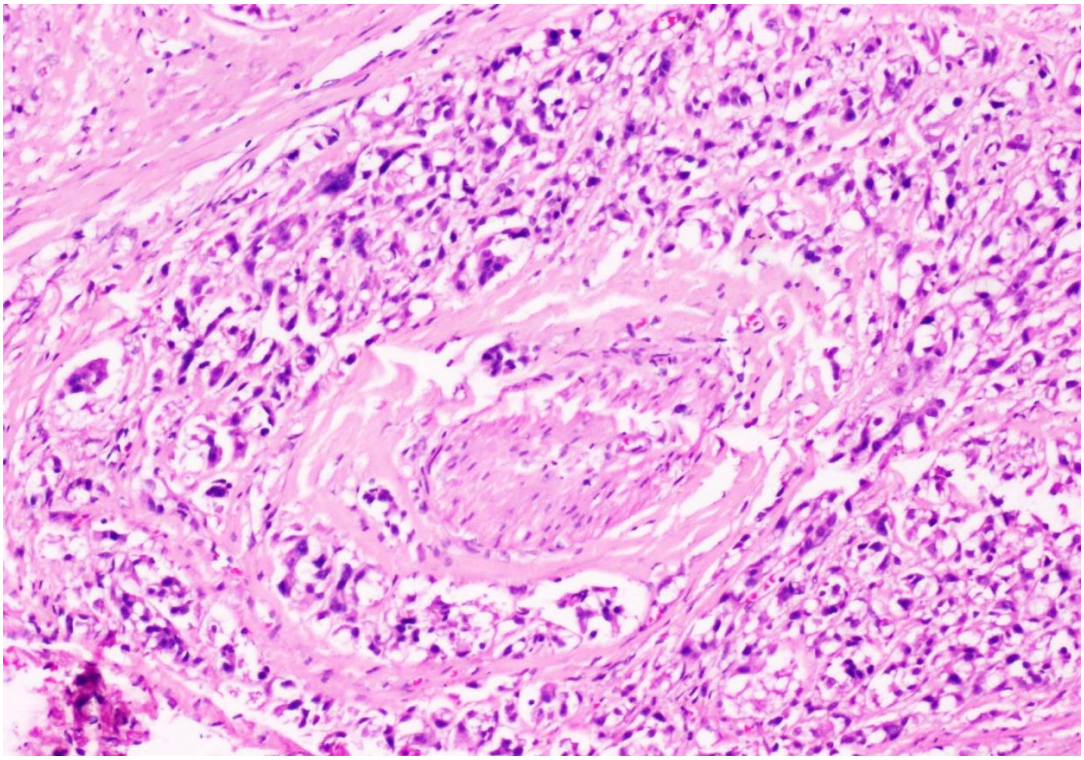


Figure 38. Perineural invasion in melanoma (H&E, x100)

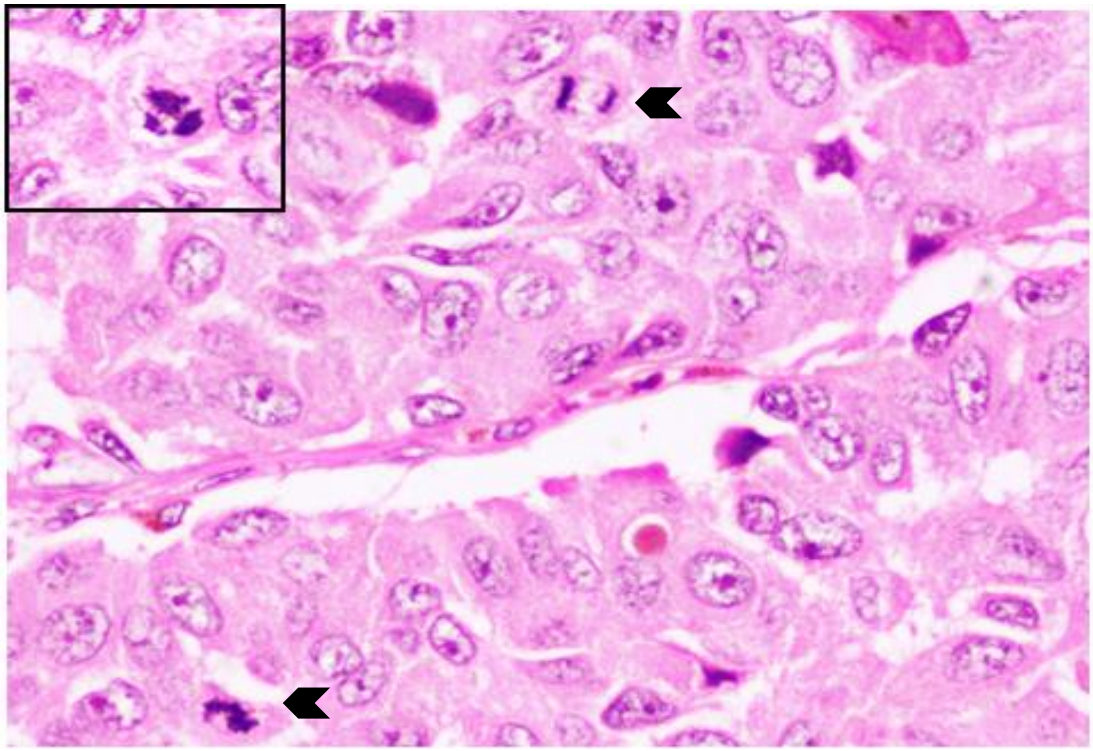


Figure 39. Mitotic activity in melanoma indicated by arrowheads, including atypical mitotic figure (inset) (H&E, x400)

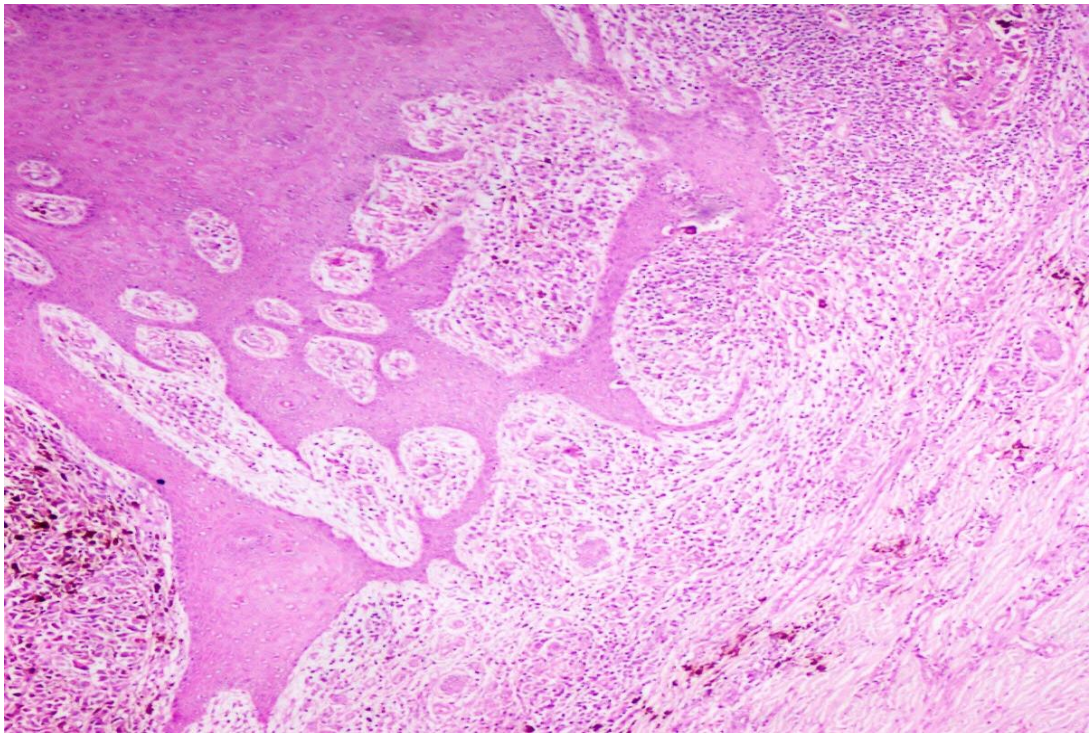


Figure 40. Regression in Melanoma (H&E, x40)

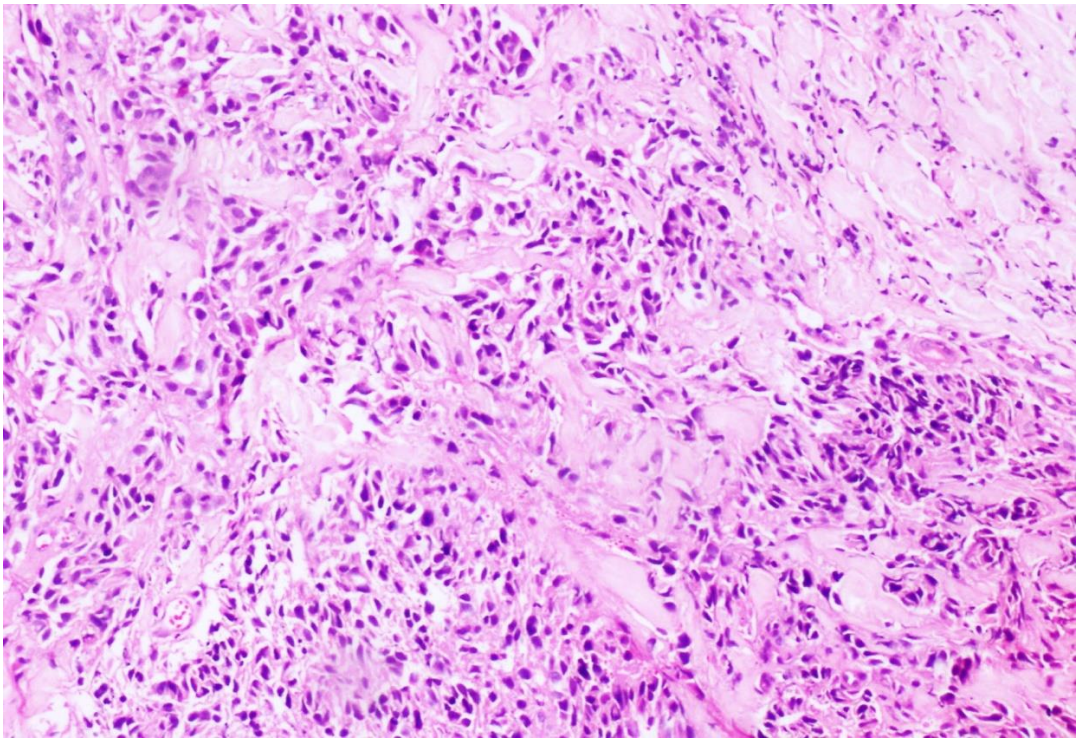


Figure 41. Absent response of tumour infiltrating lymphocytes in melanoma (H&E, x100)

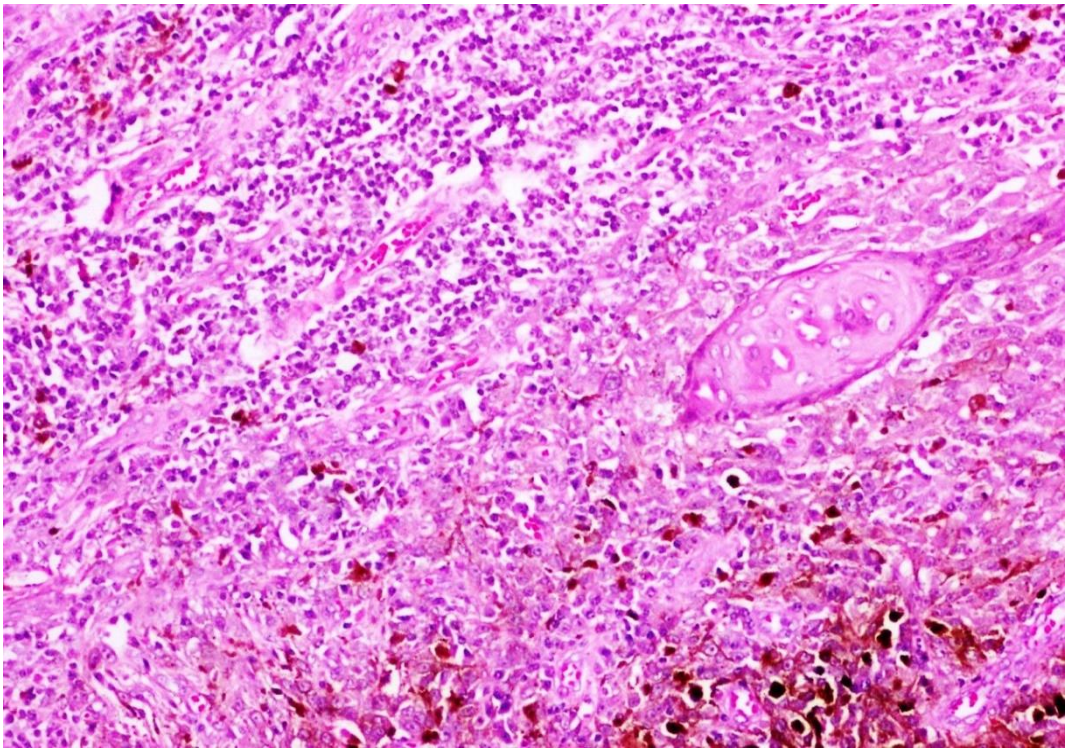


Figure 42. Brisk response of tumour infiltrating lymphocytes in melanoma (H&E, x100)

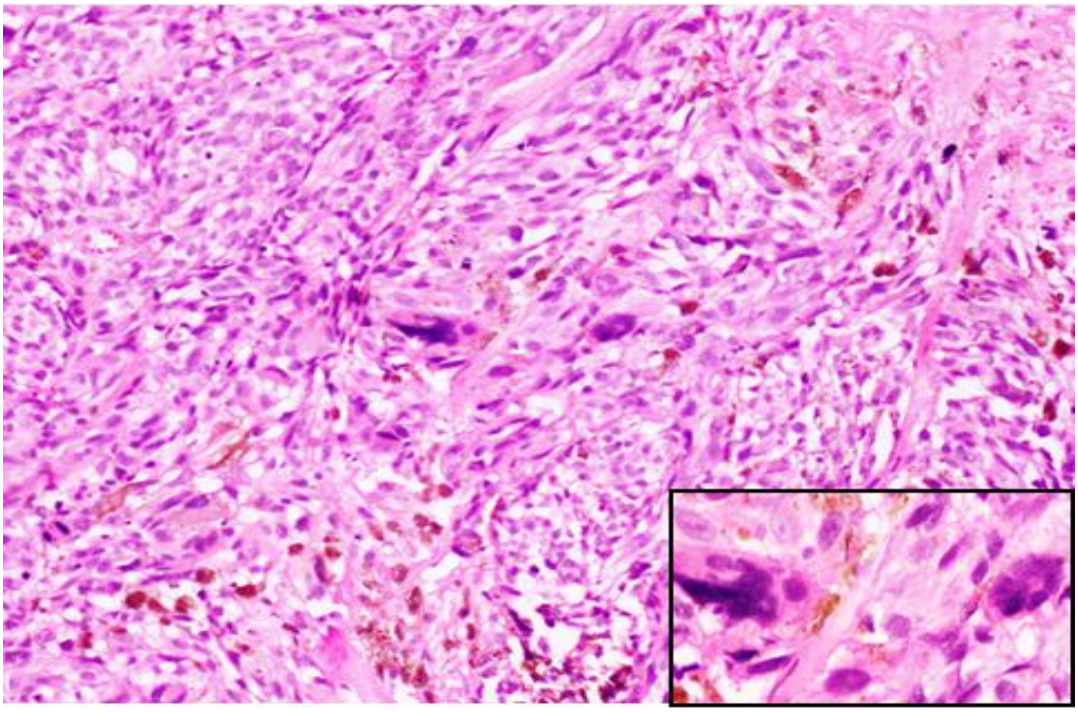


Figure 43. Giant cells in melanoma (H&E, x100); inset (x400)

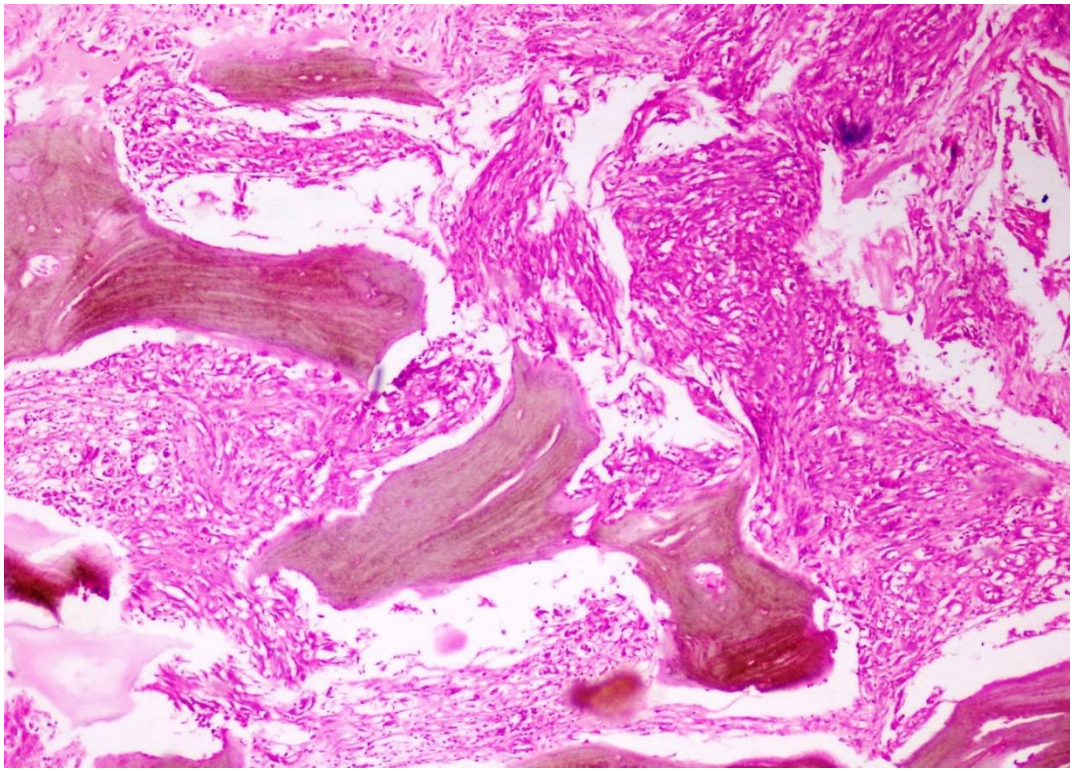


Figure 44. Bone invasion in melanoma (H&E, x40)

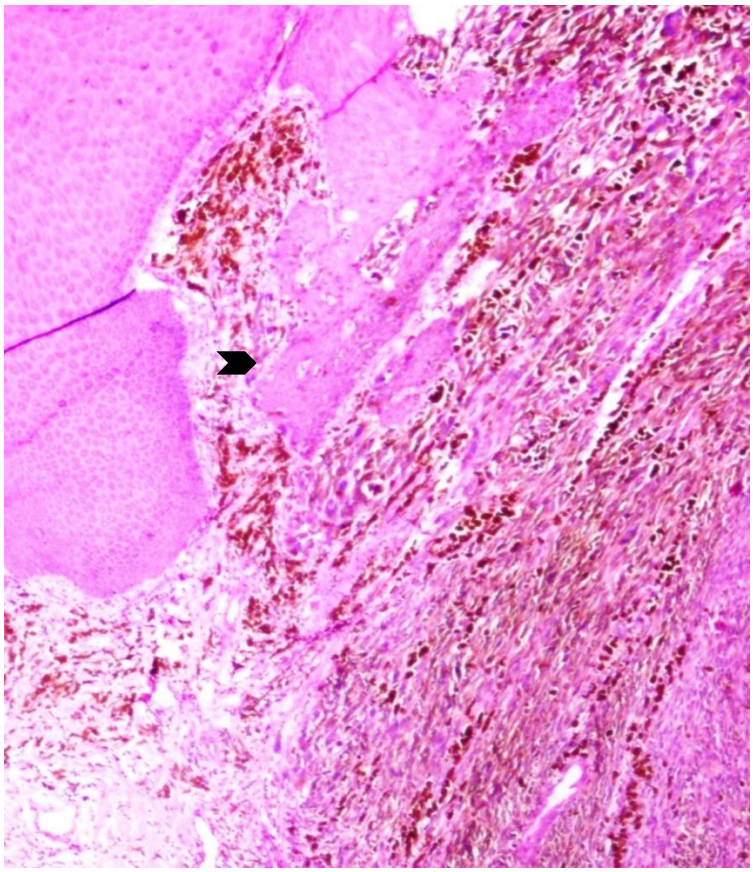


Figure 45. Abrupt circumscription in melanoma, indicated by arrowhead (H&E, x40)

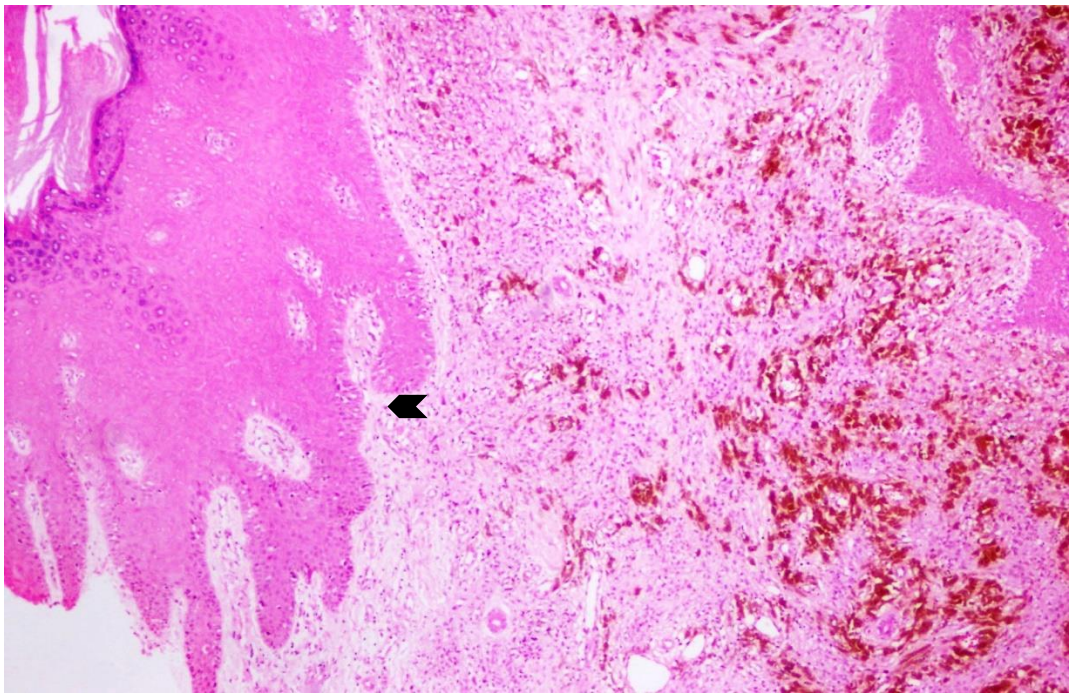


Figure 46. Continuous circumscription in melanoma, indicated by arrowhead (H&E, x40)

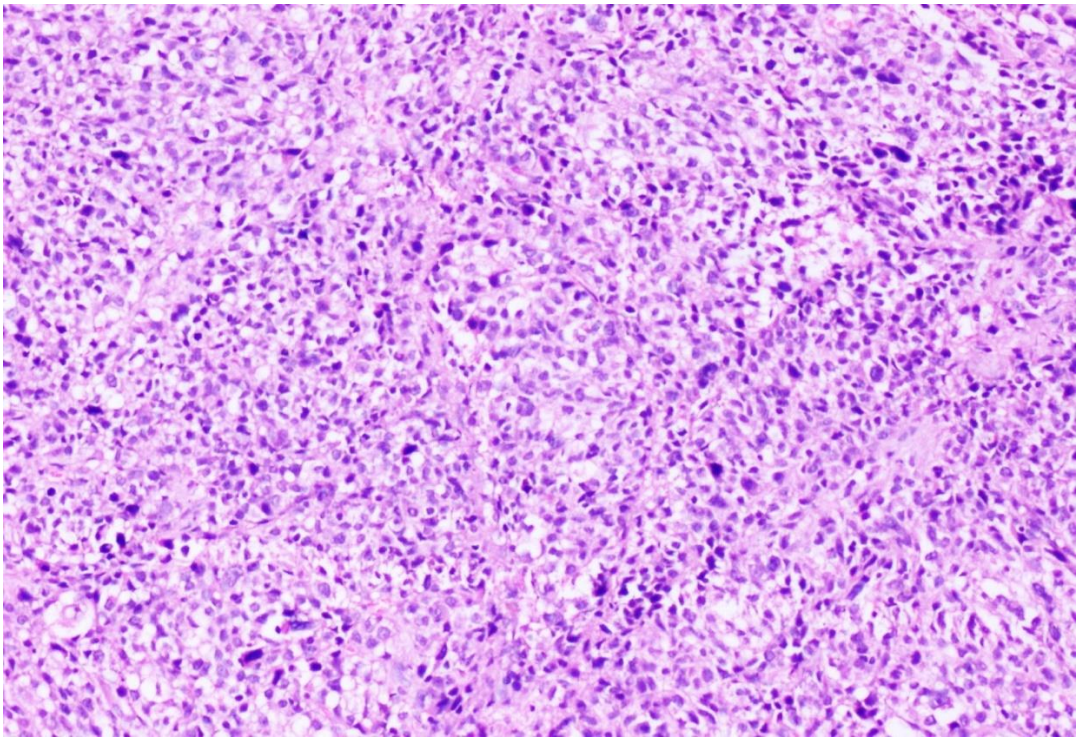


Figure 47. Epithelioid cell morphology in melanoma (H&E, x100)

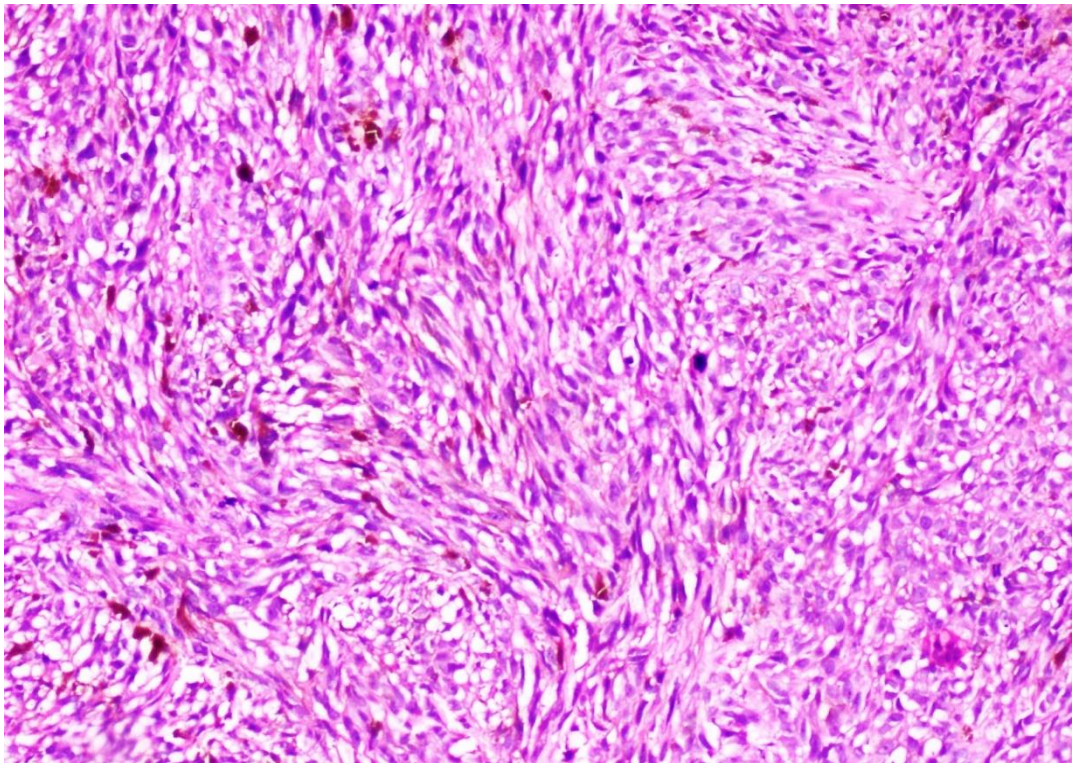


Figure 48. Spindled cell morphology in melanoma (H&E, x100)

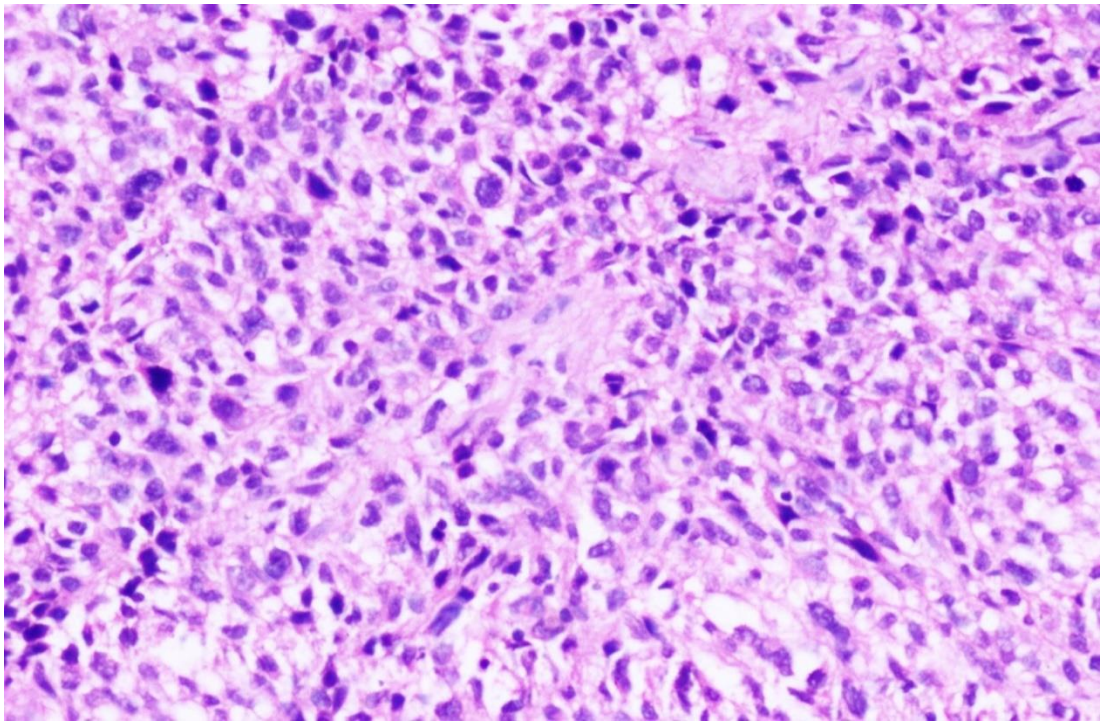


Figure 49. Medium cell size in melanoma (H&E, x200)

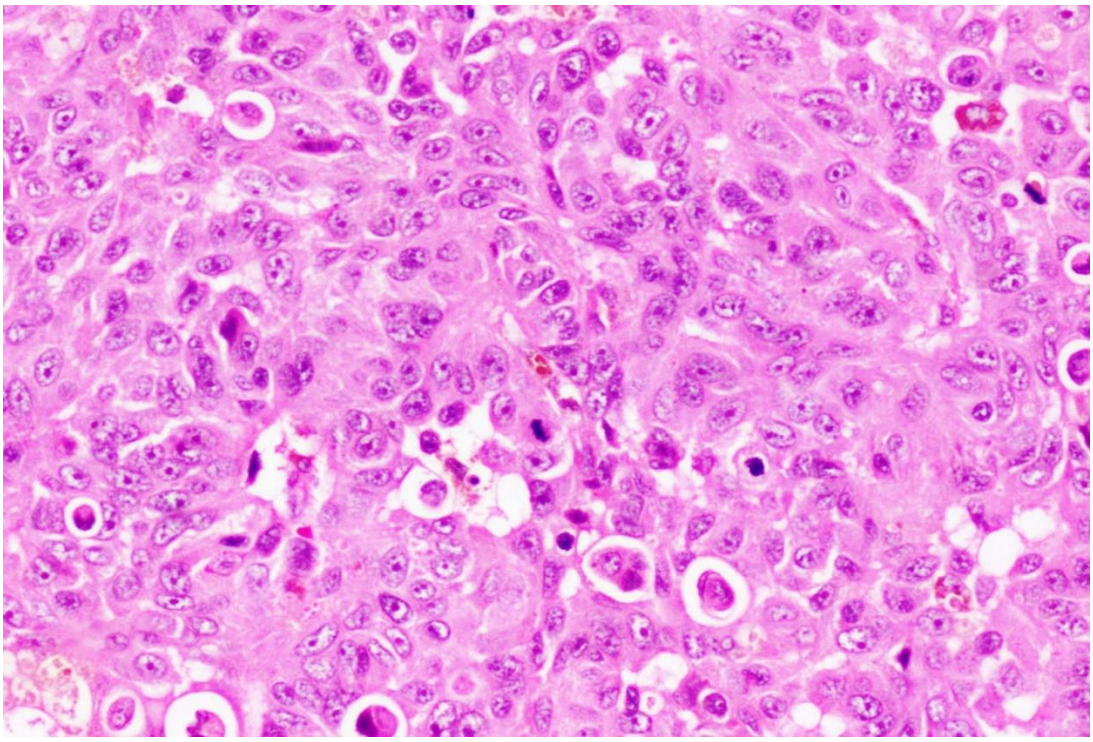


Figure 50. Large cell size in melanoma (H&E, x200)

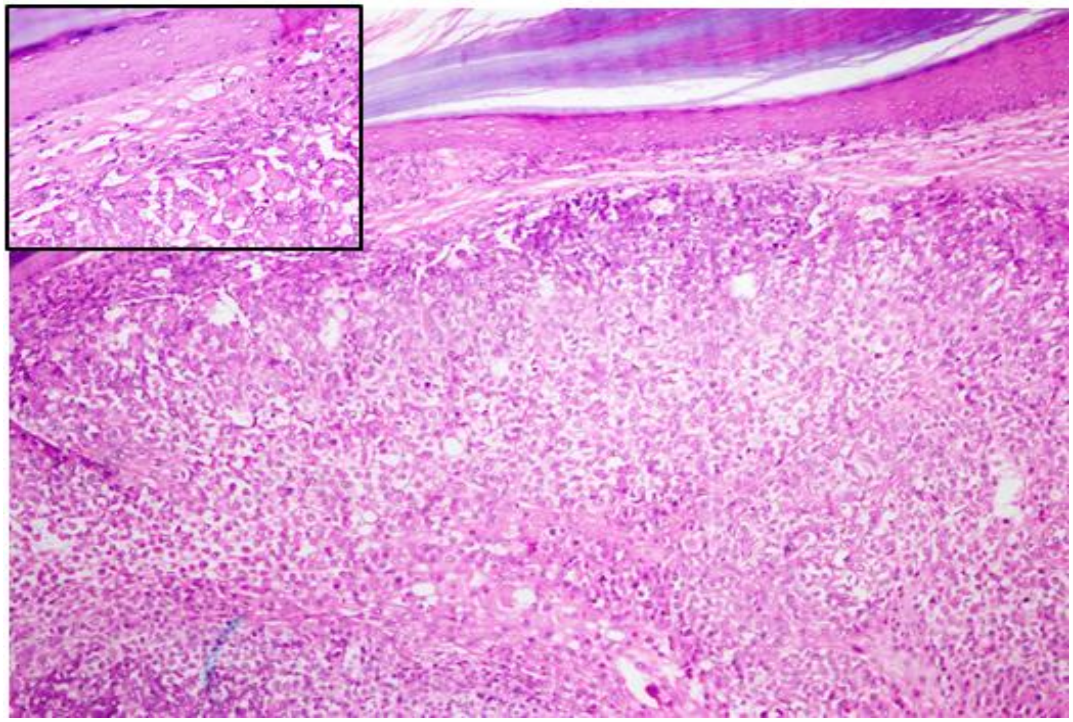


Figure 51. Satellite lesion in melanoma (H&E, x40); inset (x200)

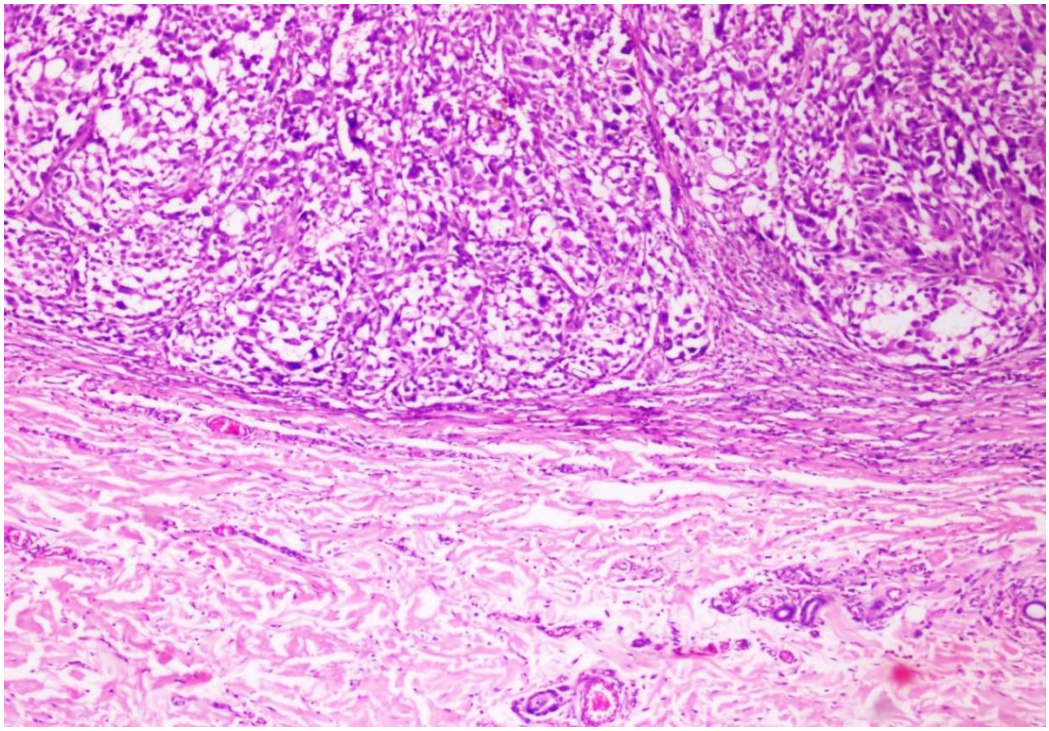


Figure 52. In transit metastases in melanoma (H&E, x40)

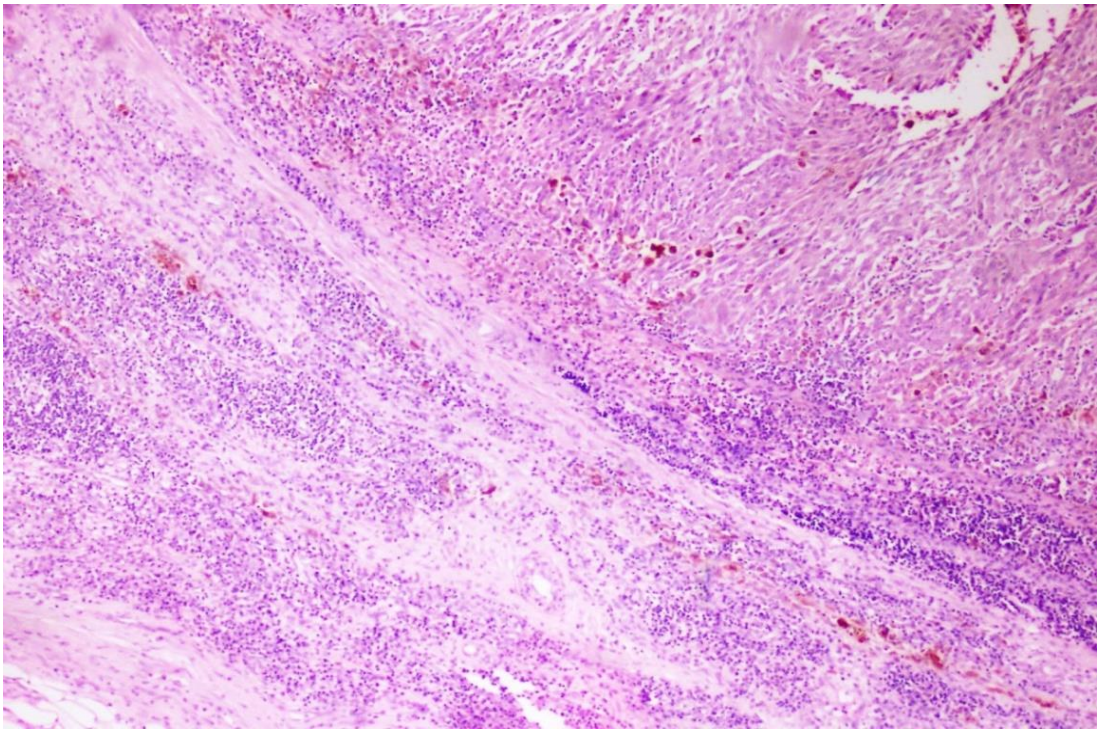


Figure 53. Lymph nodal involvement in melanoma (H&E, x40)

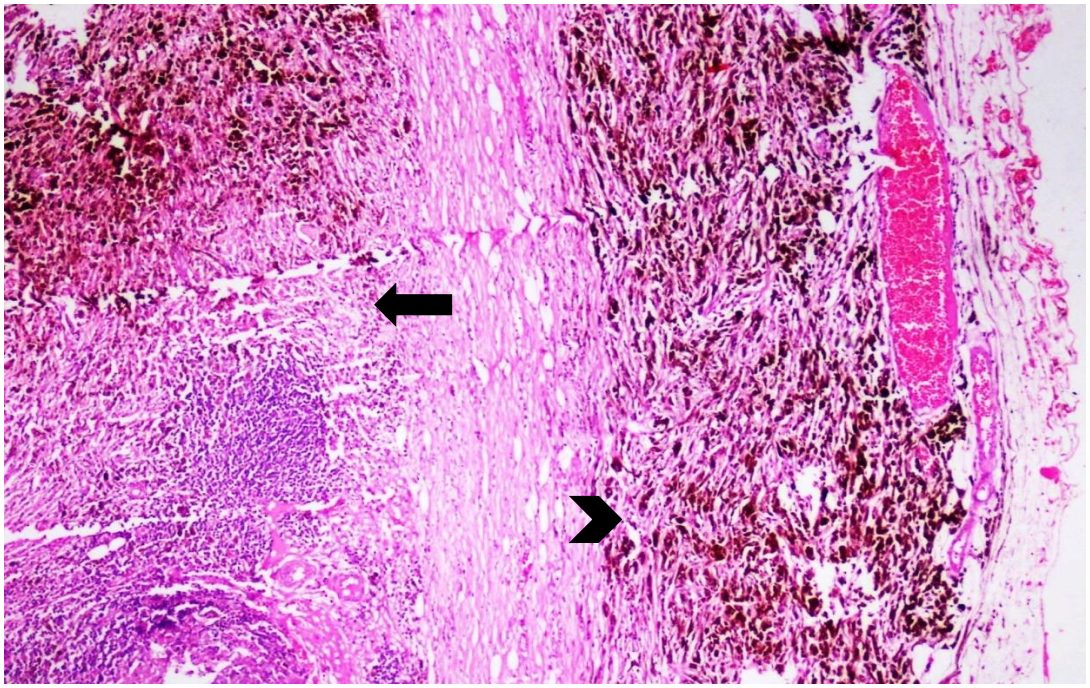


Figure 54. Metastatic tumour deposits in lymph nodes (indicated by arrow), with extracapsular extension (indicated by arrowhead) (H&E, x40)

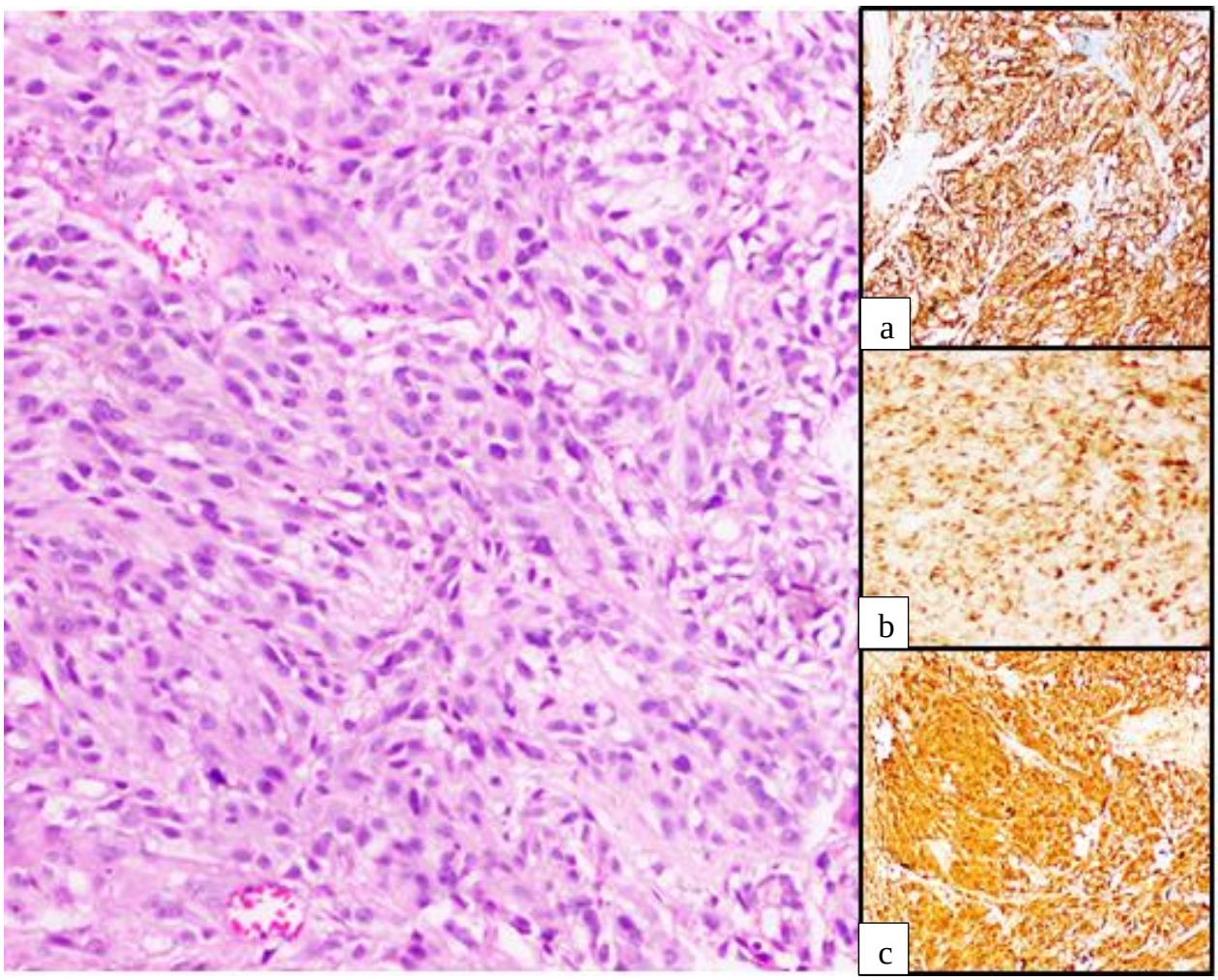


Figure 55. Immunohistochemical staining in melanoma (x100); HMB45 (a), MelanA (b) and S100 (c)

DISCUSSION

DISCUSSION:

Malignant melanoma is an aggressive and fatal form of malignancy. In our study, the patients displayed an earlier age at presentation (median: 51.5 years). Cases of cutaneous melanomas presented earlier (median: 56 years), by at least one decade when compared to the SEER data (median: 64 years) for cutaneous melanomas (5), though it correlates with many studies on Asian and Indian population by Jung et al and Radhika et al. (21,32,50) Though studies by Singer et al and Ahmad et al have shown that anal melanomas present later than cutaneous cases (peak in the seventh decade), the median age in our study was 48 years. (9,39) There was an overall male preponderance, with cutaneous melanomas having M:F ratio of 1.6:1 correlating with global studies exhibiting M:F ratio of 3:2. (5,31) While anal melanomas usually showed no sex predilection with few global studies showing an increased incidence in females (41), our study displayed a male predominance (M:F ratio of 2.6:1) which correlates with Asian studies. (40) Unlike the Caucasian population where the most common site is the trunk and back, the most commonly involved site in Asians and Indians are the palmoplantar sites and lower extremities. (32,119,146) The most common cutaneous site of involvement in our study was the foot, particularly the weight bearing portion of heel, correlating with the literature.

Acral lentiginous and nodular subtypes of melanoma together accounted for 88% of all cases, with only 2 (4%) cases of superficial spreading melanomas. We did not find any case of lentigo maligna melanoma in our study. Acral lentiginous melanomas were the most common of all cutaneous melanomas in our study, confirming the fact

that Asians had a higher incidence of acral lentiginous melanomas as compared to the Caucasian population where superficial spreading melanoma is the most common subtype of primary invasive malignant melanoma. (3,21,33) Four rare histological variants, namely pigment synthesising melanoma, rhabdoid melanoma, spitzoid melanoma and balloon cell melanoma were identified in our study apart from the commoner histopathological subtypes of invasive melanomas.

Cutaneous melanomas presented with tumours almost twice as large (median: 3.0 cm) as compared to anal melanomas (median: 1.5 cm). In a study by Massad et al, the mean size of tumour in cutaneous melanomas at the time of presentation was noted to be 2.1 cm (49) In contrast to our findings, few studies showed that anal melanomas presented with larger tumour size ranging from 2.5-5.7 cm. (40,229) However, anal melanomas were found to have greater tumour thickness (median: 7.80 mm), correlating with other global and Asian studies. (10) A large proportion of tumours in our study displayed Breslow thickness ≥ 4 mm correlating with Asian studies (33,130), as melanomas in Caucasians were usually ≤ 3 mm thick. (126,128)

Almost 96% patients in our study had Clark level of invasion 4 and 5, correlating with other Asian studies (50,133), while many tumours in Caucasian population had a Clark level of 3 and 4. (128) This might be due to the fact that majority of the tumours were in the foot in our study and therefore, asymptomatic for long periods of time. The median mitotic index was 12/ mm^2 (0-74/ mm^2) with 72% cases displaying mitotic rate of > 6/mm^2 in our study. Massad et al (49) had estimated the median

mitotic index to be 7/ mm^2 in a study of cutaneous melanomas in Asian population, whereas studies on Caucasian population showed a large proportion of tumours displaying a mitotic rate of $\leq 6/ mm^2$. (48,130,230) Two patients displayed only a radial growth phase in our study, the involved sites being vulva and foot. Almost 96% of melanomas showed vertical phase of growth, correlating with the increased prevalence of vertical phase tumours. (48–50)

About 88% of tumours displayed ulceration, correlating with the increased frequency of ulcerated tumours in Asian studies. (35,130) All anal melanomas displayed ulceration in our study, similar to the findings of Ben-Izhak et al (135,136). Lymphovascular and perineural invasion were present in 70% and 42% cases respectively in our study. Farahmand et al (130) had observed only 24% and 14% of lymphovascular and perineural invasion in a study conducted in Asian population, which is much lower than the prevalence in our study.

Satellite lesions/ in transit metastases were found in 36% patients with cutaneous melanomas in our study, with one case displaying microsatellite despite the absence of clinically detectable lesion. Studies by Kibrite et al (145) and Yu et al (146) have shown the frequency of satellitosis and in transit metastases to be 10-20%, which is less than the findings in our study. One patient who had anal melanoma was found to have a multifocal synchronous tumour. There have been case reports where such synchronous tumours have been documented in the gastrointestinal tract, but whether

to consider them as synchronous multifocal tumours or satellite lesions has not been clearly stated. (231,232)

The most common response of tumour infiltrating lymphocytes was “non brisk” (72%) in our study while absent response which is usually associated with adverse outcome was found in only 6% cases, correlating with global and Asian studies. (127,133) Regression was present in 20.5% cases of cutaneous melanomas, correlating with the findings (21.7%) of Gamsizkan et al in Asian population. (133) Regression was not identified in anal melanomas in our study.

None of the cases under study showed features of solar elastosis, while Massad et al (49) had observed an incidence of 24% solar elastosis in Asian patients with cutaneous melanomas. This is in contrast to Caucasian studies which displayed a higher incidence (70-80%) of solar elastosis. (127) Almost 76% of the tumours displayed cellular pigmentation, majority of which showed very high granular cytoplasmic pigmentation in our study, which correlated with the findings of Viros et al (82%) and Radhika et al (77%), though the latter was a study of melanomas based on morphological features in cytological preparations. (4,32)

Presence of upward scatter (82%) and nest formation (80%) of the atypical melanocytes with continuous lateral circumscription (86%) and hyperplasia (72%) of the overlying epidermis were also noted in our study. This correlated with the findings of Viros et al (4), with the exception of nature of lateral circumscription (abrupt, 40%)

in their study. Epithelioid and spindled cell morphology accounted for 64% and 36% cases respectively, correlating with literature. (49,230) Many tumours displayed large size of cell, nucleus and nucleolus (88%) in our study. Tumour giant cells were present in 12% of all cases under study, a larger proportion of which occurred in cutaneous melanomas, correlating with the study conducted by Srisuttiyakorn et al. (233) Bone invasion was present in 14.3% of cutaneous melanomas, correlating with other studies conducted in Asian population (234,235)

In keeping with the most common site of involvement being lower limb in our study, 81.8% (n=9) patients underwent block dissection of inguinal lymph nodes. The median size of lymph node was 3.3 cm (0.5-10.0 cm) with 55.6% cases measuring ≤ 3.0 cm, correlating with other studies. (236,237) Eleven patients showed metastatic tumour in lymph nodes (49,146) with extracapsular extension in 54.5% (n=6) similar to studies by Mann et al and Keenan et al. (165,238) Fine needle aspiration and cytological examination proved to be a good tool to detect metastases in clinically suspicious lymph nodes in our study (87.5%, n=7), far higher than described by Basler et al. (239)

Serum LDH levels were available only in 9 patients, of which 44.4% had elevated levels of serum LDH and all were cutaneous melanomas. One anal melanoma in which serum LDH levels were available was within the normal limits. This correlates with the observations of Ishihara et al and Ercelep et al, wherein they found 52.6% of

cutaneous melanomas showed elevated levels of serum LDH while the levels were within normal limits in 53% of mucosal melanomas. (168,176)

Adjuvant therapy was provided in 12 cases in our study, of which 75% were offered chemotherapy and 16.7% patients were provided with interferon therapy, similar to other global studies. (167,168)

Follow-up was present in 80% cases, with 10 patients completely lost to follow-up (median: 14.5 months, 2-50 months). Recurrence was present in 20% cases; anal melanomas displayed a higher frequency of recurrence, correlating with literature. (3,135,240) Metastases was present in 46% cases; majority developed metastases during the course of the disease, correlating with the findings of Sharma et al and Keenan et al (165,222) While majority of the cutaneous melanomas developed metastases during the follow-up period, a larger proportion of anal melanomas had metastases on presentation, correlating with studies by Weyandt et al and Row et al. (159,171) The most common sites involved by metastases were liver and lung. Only one patient with cutaneous melanoma had brain metastasis in our study. These findings correlate with other global and Asian studies. (172,222)

As anal melanomas did not have any standard pathological staging criteria, they were classified according to the AJCC (2010) pathological classification in this study, as maximum thickness of tumour and depth of invasion were important prognostic factors considered for staging in the earlier classifications. (9,225) Almost 74% cases

were of pathological stage T4, in keeping with the fact that majority of tumours displayed greater tumour thickness, of which 34 cases were in the pT4b category. This could be attributed to the increased frequency of ulcerated tumours in our study. These findings correlate with the observations by Gamsizkan et al and Yu et al. (133,146) In cases where lymph node staging could be assessed, 87% cases were of N2 and N3 categories, correlating with the findings of Tas et al and Keenan et al. (50,165) In a larger proportion of cases (96%), metastases was detected only clinically or with the help of radiological aids. About 83% of the patients with metastases were of clinical stage M1c. All cases of anal melanomas with clinically detectable metastases were in the cM1c category, correlating with global and Asian studies. (9,50)

When the cases were staged according to the new AJCC (8th Edition) classification (184), we found no changes in the pathological T categorisation (pT Stage) of tumours. Pathological N Stage (pN Stage) displayed a shift to left, with majority of pN2 stage tumours of 7th AJCC staging being reclassified as pN1 as per the 8th Edition AJCC Staging system **(Fig. 70)**, correlating with study by Haydu et al. (185) This shift was attributed the increased frequency of pN2c tumours with satellite lesions/in transit metastases) in our study. There was only one patient with brain metastases who would be reclassified under cM1d stage, according to the new AJCC classification.

Anal melanomas displayed a higher rate of recurrence and metastases as compared to cutaneous melanomas, correlating with Asian and global studies. (9,135,240) Anal melanomas were associated with a higher rate of metastases on presentation, while

patients with cutaneous melanomas developed metastases during the course of the disease in our study, correlating with studies by Row et al (171) and Tas et al (241). Patients with nodular melanomas displayed a higher rate of metastases on presentation in our study, while those with acral lentiginous melanomas developed metastases at a later stage. Cutaneous melanomas were associated with higher rate of single organ metastases, while anal melanomas displayed the propensity to develop metastases in multiple sites, correlating with literature. (173,174) It was noted that presence of metastases was associated with provision of adjuvant therapy, but this was inferred to be more of a consequence than as the cause, as many patients with metastases were offered palliative chemotherapy. We also found that Breslow Thickness served as a confounding factor in our study, especially with respect to distant metastases, when the overall data was analysed. Anal melanomas were observed to be a significant risk factor for metastases as compared to cutaneous melanomas (RR: 2.4, 95% CI: 1.4-4.1), especially in tumours with Breslow thickness > 4mm. **(Table 31)**

Anal melanomas were also found to be thicker tumours displaying nodular subtype and significantly associated with increased depth of invasion (Clark Level V) as compared to cutaneous melanomas. In our study, males were noted to present with ulceration and smaller tumour size of ≤ 4 cm as compared to females, the difference being statistically significant. Acral lentiginous melanomas were associated with elevated levels of serum LDH as compared to anal melanomas which predominantly showed serum levels within the normal limits, correlating with the studies of Ishihara et al and Ercelep et al. (168,176)

In cases exhibiting regression, we found a trend wherein those cases with a greater extent of regression presented with metastases on diagnosis. Similarly, melanomas with medium cell size had higher rate of metastases on presentation, as compared to melanomas with large cell size. These findings, although statistically not significant, correlate with the fact that smaller cell size and completeness of regression behave as adverse prognostic factors in melanomas. (65,242) Extranodal extension of tumour was associated with the presence of distant metastases, correlating with the findings of Keenan et al (165), though the difference was not statistically significant in our study. Anal melanomas displayed a trend towards earlier age at presentation ($\leq$ 60 years) with ulceration, similar to studies conducted by Bello et al. (135,136) Acral lentiginous melanomas showed a higher frequency of regression as compared to nodular melanomas, but this may be attributed to the site of origin as acral melanomas are more common in cutaneous sites. **(Table 4, Tables 24-27)**

We estimated the 1-year, 2-year and 3-year overall survival (OS) rates of all melanomas to be 77.8%, 22.2% and 5.6% respectively with a median OS of 16 months (95% CI: 14-35 months). Lesser OS was significantly associated with medium cell size and involved peripheral margin of invasive component in our study, correlating with global and Asian studies. (65,146,153) Factors which showed a trend towards adverse prognosis with lesser overall survival included gender (females), anal melanomas, absence of tumour infiltrating lymphocytes, increased Clark level, vertical growth phase, ulceration, amelanotic tumours, involved peripheral margin of in situ component, involved deep margin of invasive melanoma, larger metastatic

nodal size (> 6cm), presence of clinically detectable satellite lesions/ in transit metastases, elevated levels of serum LDH and absence of adjuvant therapy. **(Figs. 21-22, Figs. 71-74, Table 28)**

The 1-year, 2-year and 3-year distant metastases free survival (DMFS) rates were 64.3%, 28.6% and 14.3% respectively in our study (median DMFS: 25 months, 95% CI: 8-36 months). We found presence of satellitosis and in transit metastases to be significantly associated with lesser DMFS, correlating with other studies. (133,243) In fact, Zbytek et al (244) suggested that satellite lesions and in transit metastases were possible routes of local metastases. Other adverse prognostic factors associated with decreased metastasis free survival included gender (females), cutaneous melanomas with acral lentiginous subtype, increased Breslow thickness and Clark level, increased mitotic rate (> 12/mm^2), amelanotic tumours, medium cell size, spindled cell morphology, presence of tumour giant cells, involved margins, increased size of involved lymph node (> 6cm) with extracapsular extension and advanced stage (pT4), though the association was not statistically significant. **(Figs. 23-24, Figs. 75-77, Table 29)**

We estimated the 1-year recurrence free survival (RFS) to be 40% (mean: 29.8 months, 95% CI: 22.5-37.1 months). Recurrence was identified in only 5 patients. Factors displaying a trend towards reduced recurrence free survival included gender (females), anal melanomas with nodular subtype, increased Clark level, amelanotic tumours, epithelioid cell morphology, involved margins, satellitosis/in transit

metastases and presence of extranodal tumour extension. **(Fig. 25, Figs. 78-79, Table 30)**

Studies by Bartlett et al and Brozyna et al have shown that males and pigmented tumours display a worse prognosis in melanomas. (242,245,246) In contrast, we observed that females and absence of cellular pigmentation (amelanotic tumours) tended to have overall poor prognosis. Though many of the above mentioned factors were not associated with the development of metastases or recurrence in our study, they had an impact on the survival of patients with melanomas. Factors such as lymphovascular and perineural invasion were not found to be associated with metastases or survival outcome in our study, correlating with the findings of Egger et al and Ghaferi et al, thus implying that these parameters might not be actually significant prognostic factors in melanomas. (247,248)

Studies in Caucasian population have shown the frequency of *BRAF*^{V600E} mutation to be 40-70% (170,196,203), while Asians display a frequency of 10-40% (205,206,209) However, all the cases in which sequencing was performed after adequate DNA amplification in our study (n=45) displayed absence of *BRAF*^{V600E} mutation. **(Figs. 27-28, Figs. 80-81)** Though the prevalence of *BRAF*^{V600E} mutation in our study was 0%, the estimated probable prevalence for *BRAF*^{V600E} mutation in our population would be 2.7% (95% CI: 0.2-8.8%) by Bayesian statistics. Still, the inference of median value based on statistical concept is lower from our study, than the actual prevalence of

BRAF^{V600E} mutation reported in Asian population. Therefore, this finding might be significant in the Indian population especially for the usefulness of targeted therapy.

Viros et al (4) had developed algorithms to predict *BRAF* mutation status based on the phenotypical characteristics. They found that presence of increased upward scatter and nesting pattern of intraepidermal melanocytes with larger, rounder and more pigmented cells were predictive of *BRAF* mutation status. We found that the findings in our Indian predominant population were different from those described in the Caucasians with respect to histomorphological parameters and *BRAF* mutation status. In our study, most of the cases showed increased nesting, increased upward scatter, larger pigmented cells with epithelioid morphology and continuous lateral circumscription in the absence of *BRAF*^{V600E} mutation. This is a significant finding which should be borne in mind for future studies on the correlation of histopathological parameters and BRAF mutational status, in an Indian scenario.

CONCLUSION

CONCLUSION:

- Primary melanomas, both anal and cutaneous, present at an younger age (median 51.5 years) with acral lentiginous being the commonest subtype.
- Melanomas in our population are thicker and present at an advanced stage.
- Anal melanomas present with metastases more frequently than cutaneous melanomas, especially in multiple sites and also display higher rates of recurrence.
- Anal melanomas are a significant risk factor for the development of distant metastases.
- Nodular melanomas show a higher rate of metastases on presentation, while acral lentiginous melanomas are associated with development of metastases at a later stage in the course of the disease.
- Acral lentiginous melanomas are associated with elevated levels of serum LDH, as compared to nodular melanomas which do not show any significant difference in LDH levels.
- Significant adverse prognostic factors for overall survival include medium cell size and involved peripheral margins.
- Presence of satellite/in transit metastases is significantly associated with increased metastases and reduced survival.
- Female gender and absence of cellular pigmentation (amelanotic tumours) tend to have poor overall prognosis.

- Factors such as increased Breslow thickness and Clark level, vertical growth phase, ulceration, increased mitotic rate (> 12/mm^2), absence of tumour infiltrating lymphocytes, presence of tumour giant cells, involved margins, increased size of metastatic lymph node (> 6cm) with extracapsular extension, elevated levels of serum LDH and advanced stage are found to be associated with poor prognosis, though not statistically significant.
- Complete absence of $BRAF^{V600E}$ mutation in all the amplifiable cases, with an estimated probable prevalence of 2.7% in our population shows that the actual prevalence of $BRAF^{V600E}$ mutation in the Indian population is much lower than that described in Asian literature.
- Increased nest formation and upward scatter, larger pigmented cells with epithelioid morphology and continuous lateral circumscription are associated with absent $BRAF^{V600E}$ mutation in our population.

LIMITATIONS

LIMITATIONS:

- One of the main limitations of our study is the small sample size and short term data sets causing limitation of our results for survival and mutation analyses.
- Our study lacked the power to analyse and assess the correlation and prognostic effects of low frequency variables.
- Many patients had too short or inadequate follow-up.
- Due to monetary constraints, we were not able to include a larger population or perform further molecular analyses to look for the presence of other mutations in our population.

DIRECTIONS FOR FUTURE RESEARCH:

In our study, we performed molecular analysis only for *BRAF*^{V600E} mutation in cutaneous and anal melanomas. Other types of *BRAF* mutations as well as mutations in *NRAS, KIT* and *PIK3CA* have to be analysed in a larger population using Next Generation sequencing technologies to obtain an overall prevalence of all the mutations in our ethnicity as well as to put forth the molecular classification of melanomas in an Indian/Asian scenario. This may be followed by comparison of the mutational status with immunohistochemical studies to evaluate the expression of corresponding protein for an early diagnosis of melanoma as well as to use targeted therapy in a cost effective manner.

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BIBLIOGRAPHY

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ANNEXURES

ANNEXURES

1. CLINICAL RESEARCH FORM

PRIMARY CUTANEOUS AND ANAL MALIGNANT MELANOMA: A COMPLETE HISTOMORPHOLOGICAL STUDY WITH CLINICOPATHOLOGICAL CORRELATION AND BRAF MUTATION STATUS

Serial No: Biopsy No: Hospital No:

Age: Gender: M/F Residence:

CLINICAL DATA:

Clinical site:

Specimen type:

Wedge biopsy Mucosal biopsy Wide local excision

Resection specimen Excision biopsy Slide/Block referral

GROSS:

Tumour size:

HISTOMORPHOLOGICAL DATA:

IN SITU MELANOMA:

Not identified Present Cannot be assessed

Histopathological Subtype:

Superficial spreading Acral lentiginous Lentigo maligna

Not otherwise specified Others (Specify):

Dermal Regression:

Not identified

Present : 0-25% 25-50% 50-75% > 75%

Uncertain Cannot be assessed

Margins:

Peripheral: Involved Not involved but <1 mm

Not involved >/=1 mm ..... (to nearest 1 mm)

Uncertain Not applicable

Deep: Involved Not involved but <1 mm

Not involved >/=1 mm ..... (to nearest 1 mm)

Uncertain Not applicable

INVASIVE MELANOMA:

Absent Present

Histopathological Subtype:

Superficial spreading Acral lentiginous Lentigo maligna

Nodular Not otherwise specified Others (Specify)

Breslow Thickness: ..... (in mm)

Ulceration:

Not identified Present

Uncertain Cannot be assessed

Mitotic index: ..... /mm^2

Lymphovascular Invasion:

Not identified Present

Uncertain Cannot be assessed

Microsatellite/In transit Metastasis:

Not identified Present (Specify site)

Uncertain Cannot be assessed

Neurotropic/Perineural Invasion:

Not identified Present

Uncertain Cannot be assessed

Growth Phase:

Radial Vertical Uncertain

Tumour infiltrating lymphocytes:

Absent Non brisk Brisk

Regression:

Not identified

Present: 0-25% 25-50% 50-75% > 75%

Uncertain Cannot be assessed

(Only if pT1a/b staging not possible from mitotic index and/or ulceration):

Clark Level 4/5:

No (Specify) Yes Uncertain Cannot be assessed

Margins:

In-situ component:

Peripheral: Involved Not involved but < 1 mm

Not involved >/=1 mm ..... (to nearest 1 mm)

Uncertain Not applicable

Invasive component:

Peripheral: Involved Not involved but < 1 mm

Not involved >/=1 mm ..... (to nearest 1 mm)

Uncertain Not applicable

Deep: Involved Not involved

Not involved >/=1 mm ..... (to nearest 1 mm)

Uncertain Not applicable

Bone Invasion: Present Absent Not applicable

TNM Pathological stage T:

OTHER DEFINED MORPHOLOGICAL PARAMETERS:

Marked Solar Elastosis:

Present Absent Not applicable

Upward scatter of intraepidermal melanocytes:

Absent Slight Medium Prominent Not applicable

Nest formation of intraepidermal melanocytes:

Absent Slight Medium Prominent Not applicable

Pigmentation of melanocytes:

Absent Slight Medium High Very High

Epidermal contour:

Atrophy Thinning Normal

Thickening Hyperplasia Not applicable

Lateral circumscription:

Abrupt Continuous Discontinuous Not applicable

Cell size:

Small Medium Large

Nucleus size:

Small Medium Large

Nucleolus size:

Small Medium Large

Cell shape:

Round Ovoid Elongated Spindled

Multinucleate Tumour Giant Cells:

Present Absent

IMMUNOHISTOCHEMICAL DATA:

	Present	Absent
HMB-45:	Present	Absent
S-100:	Present	Absent
Melan A:	Present	Absent

Others (Specify):

BIOCHEMICAL DATA:

Serum LDH Levels: Present Absent

Time of Measurement:

On presentation Post-op/On follow-up Both

Sr. LDH on presentation: ..….. (in U/L)

Normal Elevated

Sr. LDH on follow-up/post op status: ..….. (in U/L)

Normal Elevated

REGIONAL LYMPH NODE DISSECTION:

Present Absent

Clinical Site:

Axillary Inguinal Others (Specify)

Localisation: Right Left Not applicable

Specimen type:

Sentinel lymph node biopsy Completion lymphadenectomy

Therapeutic lymphadenectomy

Lymph node size:

HISTOLOGICAL DATA:

Sentinel Lymph Node Biopsy:

Number of sentinel nodes identified

Number of nodes involved

For each positive node:

Location of deposit(s): Subcapsular Parenchymal

Extracapsular invasion: No Yes Uncertain Not applicable

Completion Lymphadenectomy:

Number of nodes identified

Number of nodes involved

Highest/Most apical node involved: No Yes Not identified clinically

Extracapsular invasion: No Yes Uncertain Not applicable

Margin of specimen:

Involved Not involved Uncertain Not applicable

Therapeutic Lymphadenectomy:

Number of nodes identified

Number of nodes involved

Highest/Most apical node involved: No Yes Not identified clinically

Extracapsular invasion: No Yes Uncertain Not applicable

Margin of specimen:

Involved Not involved Uncertain Not applicable

Pre-Lymph Node Dissection FNAC:

Present: Positive Negative

Absent Not applicable

TNM Pathological stage N:

OTHER CLINICAL DATA (INCL. FOLLOW-UP DATA):

Clinical Satellite: Present(Specify site) Absent

Time of Detection: On presentation On follow-up

Adjuvant Treatment: Present Absent

Type of Treatment:

Chemotherapy Interferon Both

Clinical Recurrence:

Present Absent Not applicable

Biopsy proven recurrence: Yes No

Date of Recurrence: **Recurrence Period:** ……. (in months)

Metastases: Present Absent

Onset of Metastases: On presentation On follow-up

(If Metastases on follow-up)

Date of Metastases: **Metastases Period:** ……. (in months)

Site of Metastases:

Distant skin/soft tissue (Specify site) Non regional lymph node (Specify site)

Lung Liver Adrenal

Bone Brain Others (Specify)

Biopsy proven metastases: Yes (Specify site) No

TNM Clinical stage M:

TNM Pathological stage M:

Follow-up Data: Present Absent

OPD First Visit: **OPD Last Visit:**

Last date of Follow-up: **Follow-up Period:** ……. (in months)

Survival status: Dead Alive Not known

Date of Death: **Overall Survival:** ……. (in months)

MOLECULAR DATA:

***BRAF*^{V600E} Mutation:**

Present Absent Not Amplified

2. *BRAF* MUTATION ANALYSIS

Extraction:

Formalin fixed paraffin embedded tissue blocks were used for extraction and DNA was extracted using the Nucleospin DNA FFPE Kit (Macherey-Nagel, Germany). Slides with maximum tumour tissue were selected and the corresponding areas were marked. The tissue corresponding to the marked areas on the blocks were used for DNA extraction to obtain a homogenous population of tumour cells, thereby decreasing the technical errors.

Procedure for DNA Extraction:

1) Sections of 10 µm thickness are cut from FFPE blocks & tumour tissue scraped into Eppendorf tubes containing 100 µl Lysis buffer and 10 µl Proteinase K, and incubated for a minimum of 6 hours duration at room temperature.

2) The tissue is treated with 100 µl of D-link buffer, followed by incubation in dry bath at 90^0C for 30 minutes.

3) The resultant mixture is subjected to 200 µl ethanol (centrifuged at 8000 rpm for 1 min), 500 µl wash buffer 1 (centrifuged at 11000 rcf for 1 min) followed by 500 µl wash buffer 1 (centrifuged at 11000 rcf for 2 min).

4) Then, 60 µl elution buffer is added to the above mix, followed by elution of RNA at 11000 rcf for 1 min.

The extracted samples were checked in terms of their quality and quantity using the Nanodrop (Nanodrop Technologies,USA). 50-75 ng of extracted DNA was used for the subsequent polymerase chain reaction (PCR).

Mutation Analysis: A 228 bp product was amplified in a 25 µl volume using the following reagents. **(Table 5)**

Buffer	: 2.5 µl
dNTP	: 2.0 µl
Forward primer	: 2.0 µl
Reverse primer	: 2.0 µl
Taq DNA polymerase	: 0.25 µl
Distilled water	: 15.25µl

* Extracted DNA product (1.0 µl) was added to this reagent mixture.

The following thermal cycling profile was followed for all PCRs: 95^0C for 8 min, 95^0C for 30 sec, optimized anneal for 30 sec, 72^0C for 1 min and final extension of 72^0C for 10 min. The PCR product was detected using a 2% agarose gel. Sequencing of both the sense and antisense strands of products for both primer sets was performed with an automated DNA sequencer (ABI PRISM 310 genetic analyzer) using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA).

Mutational analysis was performed by comparing the sequence with the wild type and by looking for the presence of all known mutations in this exon.

Control:

The control used for *BRAF* mutation was cell lines from ATCC-CRL-7724 (SH-4)

Table 5. Details of primers used in *BRAF* mutation analysis

Primers	**Sequence 5'-3'**
BRAF-F	**TTCATGAAGACCTCACAGTAAAAA-**3'
BRAF-R	**CCACAAAATGGATCCAGACA-3'**

3. TABLES AND FIGURES

Table 6 . AJCC 8th edition classification of Melanoma- T stage

T Classification*	Breslow thickness (in mm)	Ulceration
T1	≤ 1.00	Unknown or unspecified
T1a	< 0.80	Without ulceration
T1b	< 0.80	With ulceration
	0.80-1.00	With or without ulceration
T2	1.01-2.00	Unknown or unspecified
T2a		Without ulceration
T2b		With ulceration
T3	2.01-4.00	Unknown or unspecified
T3a		Without ulceration
T3b		With ulceration
T4	> 4.00	Unknown or unspecified
T4a		Without ulceration
T4b		With ulceration

*Tx: Primary tumour thickness cannot be assessed
T0: No evidence of primary tumour
Tis: Melanoma *in situ*

Table 7 . AJCC 8th edition classification of Melanoma- N stage

N Classification*	Number of metastatic lymph nodes	Satellite lesions/ In transit metastases
N1a	1 (clinically occult)	No
N1b	1 (clinically detected)	No
N1c	No regional lymph nodes	Yes
N2a	2-3 (clinically occult)	No
N2b	2-3 (at least 1 clinically detected)	No
N2c	1 (clinically occult or detected)	Yes
N3a	≥ 4	No
N3b	≥ 4 (at least 1 clinically detected or presence of matted nodes)	No
N3c	≥ 2 (clinically occult or clinically detected and/or presence of matted nodes)	Yes

*Nx: Regional nodes not assessed
N0: No regional metastases detected

Table 8. AJCC 8th edition classification of Melanoma- M stage

M Classification*	Site	Serum LDH
M1a	Distant skin, soft tissue including muscle and/or non-regional lymph node	Not recorded or unspecified
M1a (0)		Not elevated
M1a (1)		Elevated
M1b	Lung ± M1a sites	Not recorded or unspecified
M1b (0)		Not elevated
M1b (1)		Elevated
M1c	Non-CNS visceral sites ± M1a or M1b sites	Not recorded or unspecified
M1c (0)		Not elevated
M1c (1)		Elevated
M1d	CNS ± M1a, M1b or M1c sites	Not recorded or unspecified
M1d (0)		Not elevated
M1d (1)		Elevated

* M0: No evidence of distant metastasis
LDH: Lactate dehydrogenase

Table 9. Specimen types in Melanoma

Specimen Type	Overall (%)	Cutaneous (%)	Anal (%)
Wedge biopsy	26 (36.1)	26 (47.3)	0
Mucosal biopsy	8 (11.1)	0	8 (47.1)
Wide Local Excision	8 (11.1)	8 (14.6)	0
Resection	17 (23.6)	14 (25.5)	3 (17.7)
Excision biopsy	7 (9.7)	2 (3.6)	5 (29.4)
Slide & Block Referral	6 (8.3)	5 (9.1)	1 (5.9)
	72	**55**	**17**

Table 10. State of residence of patients with melanoma

Residence	Frequency (%)
Andhra Pradesh	1 (2)
Assam	1 (2)
Bihar	1 (2)
Chattisgarh	1 (2)
Jharkand	4 (8)
Kerala	1 (2)
Orissa	1 (2)
Tamilnadu	15 (30)
West Bengal	19 (38)
Bangladesh	1 (2)
Not known	5 (10)
Total	**50**

Table 11. Distribution of cellular pigmentation in melanoma

Cellular Pigmentation	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
Absent	12 (24)	10 (25.6)	2 (18.2)
Slight	3 (6)	3 (7.7)	0
Medium	8 (16)	3 (7.7)	5 (45.5)
High	3 (6)	3 (7.7)	0
Very high	24 (48)	20 (51.3)	4 (36.4)

Table 12. Upward scatter of intra-epidermal melanocytes in melanoma

Upward Scatter	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
Absent	7 (14)	4 (10.3)	3 (27.3)
Slight	28 (56)	23 (59)	5 (45.4)
Medium	9 (18)	8 (20.5)	1 (9.1)
Prominent	4 (8)	4 (10.3)	0
NA	2 (4)	0	2 (18.2)

NA- Not Applicable

Table 13. Nest formation of intra-epidermal melanocytes in melanoma

Nest Formation	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
Absent	8 (16)	5 (12.8)	3 (27.3)
Slight	28 (56)	23 (59)	5 (45.4)
Medium	11 (2)	10 (25.6)	1 (9.1)
Prominent	1 (2)	1 (2.6)	0
NA	2 (4)	0	2 (18.2)

NA- Not Applicable

Table 14. Lateral circumscription of intra-epidermal melanocytes in melanoma

Lateral Circumscription	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
Abrupt	2 (4)	2 (5.1)	0
Continuous	43 (86)	36 (92.3)	7 (63.6)
Discontinuous	0	0	0
NA	5 (10)	1 (2.)	4 (36.4)

NA- Not Applicable

Table 15. Epidermal contour in melanoma

Epidermal Contour	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
Atrophy	1 (2)	0	1 (9.1)
Thinning	3 (6)	1 (2.6)	2 (18.2)
Normal	5 (10)	2 (5.1)	3 (27.3)
Thickening	3 (6)	2 (5.1)	1 (9.1)
Hyperplasia	36 (72)	34 (87.2)	2 (18.2)
NA	2 (4)	0	2 (18.2)

NA- Not Applicable

Table 16. Cell shape of atypical melanocytes in melanoma

Cell shape	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
Round	22 (44)	16 (41)	6 (54.5)
Ovoid	10 (20)	7 (18)	3 (27.3)
Elongated	2 (4)	1 (2.5)	1 (9.1)
Spindled	16 (32)	15 (38.5)	1 (9.1)

Table 17. Site of lymph node dissection in melanoma

Site of Lymph Node Dissection	Overall (%)	Cutaneous (%)	Anal (%)
Axillary	2 (14.3)	2 (18.2)	0
Inguinal	5 (35.7)	5 (45.4)	0
Perirectal	3 (21.4)	0	3 (100)
Inguinal & Others*	4 (28.6)	4 (36.4)	0
	14	**11**	**3**

*Others include iliac and obturator lymph nodes

Table 18. Distribution by site of metastases in melanoma

Site of metastases	Overall (%)		Cutaneous (%)		Anal (%)	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
Distant skin/ Soft tissue	2 (8.7)	21 (91.3)	2 (14.3)	12 (85.7)	0	9 (100)
Non-regional Lymph Node	7 (31.8)	15 (68.2)	3 (23.1)	10 (76.9)	4 (44.4)	5 (55.6)
Lung	10 (43.5)	13 (56.5)	6 (42.9)	8 (57.1)	4 (44.4)	5 (55.6)
Liver	16 (69.6)	7 (30.4)	7 (50)	7 (50)	9 (100)	0
Adrenal	2 (8.7)	21 (91.3)	1 (7.1)	13 (92.9)	1 (11.1)	8 (88.9)
Bone	7 (30.4)	16 (69.6)	3 (21.4)	11 (78.6)	4 (44.4)	5 (55.6)
Brain	1 (4.4)	22 (95.6)	1 (7.1)	13 (92.9)	0	9 (100)
Others*	3 (13)	20 (87)	3 (21.4)	11 (78.6)	0	9 (100)
	23		**14**		**9**	

* Other sites of metastases include pancreas, kidney, spleen and hepatic flexure of colon

Table 19. Distribution by pT stage in melanoma – AJCC 7th edition classification

pT Stage	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
T1a	1 (2)	1 (2.6)	0
T1b	0	0	0
T2a	0	0	0
T2b	2 (4)	1 (2.6)	1 (9.1)
T3a	1 (2)	1 (2.6)	0
T3b	9 (18)	6 (15.4)	3 (27.3)
T4a	3 (6)	3 (7.7)	0
T4b	34 (68)	27 (69.2)	7 (63.6)

Table 20. Distribution by pN stage in melanoma – AJCC 7th edition classification

pN Stage	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
N1a	0	0	0
N1b	2 (4)	2 (5.1)	0
N2a	0	0	0
N2b	0	0	0
N2c	11 (22)	11 (28.2)	0
N3	10 (20)	8 (20.5)	2 (18.2)
Nx	26 (52)	18 (46.2)	8 (72.7)
N0	1 (2)	0	1 (9.1)

Table 21. Distribution by cM stage in melanoma – AJCC 7th edition classification

cM Stage	Overall (%) (n=50)	Cutaneous (%) (n=39)	Anal (%) (n=11)
M1a	2 (4)	2 (5.1)	0
M1b	2 (4)	2 (5.1)	0
M1c	19 (38)	10 (25.7)	9 (81.8)
M0	27 (54)	25 (64.1)	2 (18.2)

Table 22. Distribution of clinicopathological parameters by cutaneous site

Characteristics	Cutaneous Site		
	Finger (n=4)	**Leg/Forearm (n=3)**	**Foot (n=31)**
Age (in years)			
≤ 50	2 (50)	2 (66.7)	12 (38.7)
51-60	0	0	11 (35.5)
> 60	2 (50)	1 (33.3)	8 (25.8)
Gender			
Male	4 (100)	1 (33.3)	19 (61.3)
Female	0	2 (66.7)	12 (38.7)
Max Tumour Size			
≤ **2 cm**	1 (25)	1 (33.3)	12 (38.7)
2-4 cm	2 (50)	0	14 (45.2)
> 4 cm	1 (25)	2 (66.7)	5 (16.1)
Invasive Subtype			
SSM	0	0	2 (6.4)
ALM	2 (50)	0	19 (61.3)
Nodular	1 (25)	3 (100)	7 (22.6)
Others	1 (25)	0	3 (9.7)
Breslow Thickness (in mm)			
≤ **4.00**	0	1 (33.3)	7 (22.6)
> 4.00	4 (100)	2 (66.7)	24 (77.4)
Clark Level			
3	0	0	1 (3.2)
4	4 (100)	2 (66.7)	13 (41.9)
5	0	1 (33.3)	17 (54.9)
Growth Phase			
Radial	0	0	1 (3.2)
Vertical	4 (100)	3 (100)	30 (96.8)
Ulceration			
Absent	0	1 (33.3)	4 (12.9)
Present	4 (100)	2 (66.7)	27 (87.1)
Lymphovascular Invasion			
Absent	1 (25)	1 (33.3)	8 (25.8)
Present	3 (75)	2 (66.7)	23 (74.2)
Perineural Invasion			
Absent	3 (75)	2 (66.7)	16 (51.6)
Present	1 (25)	1 (33.3)	15 (48.4)
Tumour Infiltrating Lymphocytes			
Absent	0	1 (33.3)	2 (6.5)
Non brisk	4 (100)	2 (66.7)	21 (67.7)
Brisk	0	0	8 (25.8)
Regression			
Absent	4 (100)	3 (100)	23 (74.2)
Present	0	0	8 (25.8)
Extent of Regression			
0-25%	0	0	6 (75)
25-50%	0	0	2 (25)
Cellular Pigmentation			
Absent	2 (50)	0	8 (25.8)
Present	2 (50)	3 (100)	23 (74.2)
Upward Scatter			
Absent	0	0	4 (12.9)
Present	4 (100)	3 (100)	27 (87.1)
Nest Formation			
Absent	1 (25)	2 (66.7)	2 (6.5)
Present	3 (75)	1 (33.3)	29 (93.5)

Lateral Circumscription			
Abrupt	0	0	2 (6.5)
Continuous	4 (100)	3 (100)	28 (90.3)
NA	0	0	1 (3.2)
Cell Size			
Medium	0	0	3 (9.7)
Large	4 (100)	3 (100)	28 (90.3)
Cell Shape			
Epithelioid	2 (50)	2 (66.7)	18 (58.1)
Spindled	2 (50)	1 (33.3)	13 (41.9)
Clinical Satellite/In transit metastases			
Absent	4 (100)	1 (33.3)	19 (61.3)
Present	0	2 (66.7)	12 (38.7)
Max Lymph Node Size			
≤ 3 cm	0	1 (100)	2 (33.3)
3-6 cm	1 (50)	0	3 (50)
> 6 cm	1 (50)	0	1 (16.7)
Extranodal Tumour Extension			
Absent	1 (50)	1 (100)	2 (33.3)
Present	1 (50)	0	4 (66.7)
pT Stage			
T2	0	1 (33.3)	0
T3	0	0	7 (22.6)
T4	4 (100)	2 (66.7)	24 (77.4)
pN Stage			
N1	0	0	2 (6.4)
N2	0	1 (33.3)	10 (32.3)
N3	2 (50)	1 (33.3)	5 (16.1)
Nx	2 (50)	1 (33.3)	14 (45.2)
pN Stage (8^{th} Ed)			
N1	0	1 (33.3)	12 (38.7)
N3	2 (50)	1 (33.3)	5 (16.1)
Nx	2 (50)	1 (33.3)	14 (45.2)
cM Stage			
M1a	0	1 (33.3)	1 (3.2)
M1b	0	0	2 (6.5)
M1c	1 (25)	0	8 (25.8)
M0	3 (75)	2 (66.7)	20 (64.5)
Adjuvant Treatment			
Absent	4 (100)	2 (66.7)	26 (83.9)
Present	0	1 (33.3)	5 (16.1)
Serum LDH Levels			
Normal	0	1 (100)	3 (50)
Elevated	1 (100)	0	3 (50)
Recurrence			
Absent	3 (100)	2 (100)	13 (86.7)
Present	0	0	2 (13.3)
Metastases			
Absent	3 (75)	2 (66.7)	20 (64.5)
Present	1 (25)	1 (33.3)	11 (35.5)

* All cases not included
Abbreviations: SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, LDH – Lactate Dehydrogenase, NA – Not Applicable ($p < 0.05$ significant)

Table 23. Univariate analysis of clinicopathological parameters with recurrence and metastases

Characteristics	Recurrence		p value	Metastases		p value
	Present (n=5)	Absent (n=20)		Present (n=23)	Absent (n=27)	
Age (in years)			0.175			0.931
≤ 50	4 (80)	8 (40)		12 (52.2)	12 (44.4)	
51-60	1 (20)	4 (20)		6 (26.1)	9 (33.3)	
> 60	0	8 (40)		5 (21.7)	6 (22.2)	
Gender			> 0.99			0.559
Male	3 (60)	13 (65)		16 (69.6)	16 (59.3)	
Female	2 (40)	7 (35)		7 (30.4)	11 (40.7)	
Clinical Site			**0.038**			**0.014**
Cutaneous	2 (40)	18 (90)		14 (60.9)	25 (92.6)	
Anal	3 (60)	2 (10)		9 (39.1)	2 (7.4)	
Max Tumour Size			> 0.99			0.934
≤ 2 cm	1 (20)	3 (15)		10 (43.5)	12 (44.4)	
2-4 cm	3 (60)	12 (60)		9 (39.1)	9 (33.3)	
> 4 cm	1 (20)	5 (25)		4 (17.4)	6 (22.2)	
Invasive Subtype			0.471			0.086
SSM	0	1 (5)		0	2 (7.4)	
ALM	1 (20)	8 (40)		10 (43.5)	12 (44.4)	
Nodular	4 (80)	7 (35)		13 (56.5)	9 (33.3)	
Others	0	4 (20)		0	4 (14.8)	
Breslow Thickness (in mm)			> 0.99			> 0.99
≤ 4.00	0	2 (10)		5 (21.7)	7 (25.9)	
> 4.00	5 (100)	18 (90)		18 (78.3)	20 (74.1)	
Clark Level*			0.615			0.249
4	1 (20)	9 (45)		7 (31.8)	13 (50)	
5	4 (80)	11 (55)		15 (68.2)	13 (50)	
Growth Phase			0.200			0.207
Radial	1 (20)	0		2 (8.7)	0	
Vertical	4 (80)	20 (100)		21 (91.3)	27 (100)	
Ulceration			0.367			> 0.99
Absent	1 (20)	1 (5)		3 (13)	3 (11.1)	
Present	4 (80)	19 (95)		23 (87)	24 (88.9)	
Lymphovascular Invasion			> 0.99			0.548
Absent	1 (20)	3 (15)		8 (34.8)	7 (25.9)	
Present	4 (80)	17 (85)		15 (65.2)	20 (74.1)	
Perineural Invasion			> 0.99			0.778
Absent	2 (40)	9 (45)		14 (60.9)	15 (55.6)	
Present	3 (60)	11 (55)		9 (39.1)	12 (44.4)	
TIL			0.635			> 0.99
Absent	0	1 (5)		1 (4.4)	2 (7.4)	
Non brisk	5 (100)	14 (70)		17 (73.9)	19 (70.4)	
Brisk	0	5 (25)		5 (21.7)	6 (22.2)	
Regression			0.549			0.711
Absent	5 (100)	16 (80)		20 (87)	22 (81.5)	
Present	0	4 (20)		3 (13)	5 (18.5)	
Extent of Regression			--------			> 0.99
0-25%	0	4 (100)		2 (66.7)	4 (80)	
25-50%	0	0		1 (33.3)	1 (20)	
Mitotic Index (per mm^2)			0.794			0.508
≤ 6	1 (20)	4 (20)		6 (26.1)	8 (29.6)	
6-12	2 (40)	4 (20)		4 (17.4)	8 (29.6)	

> 12	2 (40)	12 (60)		13 (56.5)	11 (40.7)	
Cellular Pigmentation			> 0.99			> 0.99
Absent	1 (20)	6 (30)		6 (26.1)	6 (22.2)	
Present	4 (80)	14 (70)		17 (73.9)	21 (77.8)	
Upward Scatter*			> 0.99			0.687
Absent	0	1 (5.3)		4 (18.2)	3 (11.5)	
Present	5 (100)	18 (94.7)		18 (81.8)	23 (88.5)	
Nest Formation*			> 0.99			> 0.99
Absent	1 (20)	5 (26.3)		4 (18.2)	4 (15.4)	
Present	4 (80)	14 (73.7)		18 (81.8)	22 (84.6)	
Lateral Circumscription*			> 0.99			> 0.99
Abrupt	0	1 (5.6)		1 (5)	1 (4)	
Continuous	5 (100)	17 (94.4)		19 (95)	24 (96)	
Cell Size			> 0.99			0.395
Medium	0	3 (15)		4 (17.4)	2 (7.4)	
Large	5 (100)	17 (85)		19 (82.6)	25 (92.6)	
Cell Shape			0.623			0.771
Epithelioid	3 (60)	8 (40)		14 (60.9)	18 (66.7)	
Spindled	2 (40)	12 (60)		9 (39.1)	9 (33.3)	
Tumour Giant Cells			> 0.99			0.395
Absent	4 (80)	16 (80)		19 (82.6)	25 (92.6)	
Present	1 (20)	4 (20)		4 (17.4)	2 (7.4)	
Insitu Peripheral Margin[†]			> 0.99			> 0.99
Involved/ < 1 mm	2 (50)	7 (43.8)		7 (50)	10 (50)	
1-10 mm	2 (50)	9 (56.2)		7 (50)	10 (50)	
Invasive Peripheral Margin[†]			0.225			0.646
Involved/ < 1 mm	1 (25)	1 (5.9)		3 (21.4)	7 (33.3)	
1-20 mm	3 (75)	11(64.7)		9 (64.3)	10 (47.6)	
> 20 mm	0	5 (29.4)		2 (14.3)	4 (19.1)	
Invasive Deep Margin[†]			0.576			> 0.99
Involved/ < 1 mm	2 (50)	2 (28.6)		4 (57.1)	7 (50)	
≥ 1 mm	2 (50)	5 (71.4)		3 (42.9)	7 (50)	
Clinical Satellite/ITM			> 0.99			0.361
Absent	3 (60)	12 (60)		15 (65.2)	21 (77.8)	
Present	2 (40)	8 (40)		8 (34.8)	6 (22.2)	
Max Lymph Node Size			0.286			0.437
≤ 3 cm	3 (100)	2 (33.3)		3 (50)	2 (40)	
3-6 cm	0	2 (33.3)		1 (16.7)	3 (60)	
> 6 cm	0	2 (33.3)		2 (33.3)	0	
Extranodal Extension			> 0.99			0.080
Absent	1 (33.3)	3 (50)		1 (16.7)	4 (80)	
Present	2 (66.7)	3 (50)		5 (83.3)	1 (20)	
pT Stage*			> 0.99			> 0.99
T3	0	2 (10)		5 (22.7)	5 (20)	
T4	5 (100)	18 (90)		17 (77.3)	20 (80)	
pN Stage*			0.569			0.670
N2	1 (25)	7 (58.3)		5 (45.5)	6 (60)	
N3	3 (75)	5 (41.7)		6 (54.5)	4 (40)	
pN Stage (8th Ed)*			0.294			0.414
N1	1 (25)	8 (61.5)		5 (45.5)	8 (66.7)	
N3	3 (75)	5 (38.5)		6 (54.5)	4 (33.3)	
No of Sites with Distant Metastases			0.580			--------
Single	1 (25)	5 (50)				
Multiple	3 (75)	5 (50)				
Adjuvant Treatment			0.312			**0.006**
Absent	2 (40)	14 (70)		13 (56.5)	25 (92.6)	

Present	3 (60)	6 (30)		10 (43.5)	2 (7.4)	
Type of Adjuvant therapy			0.083			0.455
Chemotherapy	1 (33.3)	5 (83.3)		8 (80)	1 (50)	
IFN	2 (66.7)	0		1 (10)	1 (50)	
Chemo & IFN	0	1 (16.7)		1 (10)	0	
Serum LDH Levels			> 0.99			> 0.99
Normal	2 (66.7)	3 (50)		4 (50)	1 (100)	
Elevated	1 (33.3)	3 (50)		4 (50)	0	

* All cases not included
† Data not available for all cases
Abbreviations: SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, TIL – Tumour Infiltrating Lymphocytes, ITM – In transit metastases, IFN – Interferon, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 24. Univariate analysis of clinicopathological parameters with onset of metastases

Characteristics	**Onset of Metastases**		**p value**
	On presentation (n=9)	**On follow-up (n=14)**	
Age (in years)			0.166
≤ 50	6 (66.7)	6 (42.9)	
51-60	3 (33.3)	3 (21.4)	
> 60	0	5 (35.7)	
Gender			0.363
Male	5 (55.6)	11 (78.6)	
Female	4 (44.4)	3 (21.4)	
Clinical Site			**0.007**
Cutaneous	2 (22.2)	12 (85.7)	
Anal	7 (77.8)	2 (14.3)	
Max Tumour Size			**0.003**
≤ 2 cm	7 (77.8)	3 (21.4)	
2-4 cm	0	9 (64.3)	
> 4 cm	2 (22.2)	2 (14.3)	
Invasive Subtype			**0.029**
ALM	1 (11.1)	9 (64.3)	
Nodular	8 (88.9)	5 (35.7)	
Breslow Thickness (in mm)			0.343
≤ 4.00	3 (33.3)	2 (14.3)	
> 4.00	6 (66.7)	12 (85.7)	
Clark Level*			0.165
4	1 (11.1)	6 (46.2)	
5	8 (88.9)	7 (53.8)	
Growth Phase			0.502
Radial	0	2 (14.3)	
Vertical	9 (100)	12 (85.7)	
Ulceration			0.253
Absent	0	3 (21.4)	
Present	9 (100)	11 (78.6)	
Lymphovascular Invasion			> 0.99
Absent	3 (33.3)	5 (35.7)	
Present	6 (66.7)	9 (64.3)	
Perineural Invasion			> 0.99
Absent	6 (66.7)	8 (57.1)	
Present	3 (33.3)	6 (42.9)	
Tumour Infiltrating Lymphocytes			> 0.99
Absent	0	1 (7.1)	
Non brisk	7 (77.8)	10 (71.5)	
Brisk	2 (22.2)	3 (21.4)	
Regression			> 0.99

Absent	8 (88.9)	12 (85.7)	
Present	1 (11.1)	2 (14.3)	
Extent of Regression			0.333
0-25%	0	2 (100)	
25-50%	1 (100)	0	
Mitotic Index (per mm^2)			> 0.99
≤ 6	2 (22.2)	4 (28.6)	
6-12	2 (22.2)	2 (14.3)	
> 12	5 (55.6)	8 (57.1)	
Cellular Pigmentation			0.643
Absent	3 (33.3)	3 (21.4)	
Present	6 (66.7)	11 (78.6)	
Upward Scatter*			**0.010**
Absent	4 (50)	0	
Present	4 (50)	14 (100)	
Nest Formation*			0.602
Absent	2 (25)	2 (14.3)	
Present	6 (75)	12 (85.7)	
Lateral Circumscription*			> 0.99
Abrupt	0	1 (7.1)	
Continuous	6 (100)	13 (92.9)	
Cell Size			0.260
Medium	3 (33.3)	1 (7.1)	
Large	6 (66.7)	13 (92.9)	
Cell Shape			0.228
Epithelioid	7 (77.8)	7 (50)	
Spindled	2 (22.2)	7 (50)	
Tumour Giant Cells			> 0.99
Absent	8 (88.9)	11 (78.6)	
Present	1 (11.1)	3 (21.4)	
Insitu Peripheral Margin[†]			0.462
Involved/ < 1 mm	2 (100)	5 (41.7)	
1-10 mm	0	7 (58.3)	
Invasive Peripheral Margin[†]			0.604
Involved/ < 1 mm	1 (50)	2 (16.7)	
1-20 mm	1 (50)	8 (66.7)	
> 20 mm	0	2 (16.7)	
Invasive Deep Margin[†]			0.429
Involved/ < 1 mm	2 (100)	2 (40)	
≥ 1 mm	0	3 (60)	
Clinical Satellite/In transit metastases			0.086
Absent	8 (88.9)	7 (50)	
Present	1 (11.1)	7 (50)	
Max Lymph Node Size			0.167
≤ 3 cm	0	3 (60)	
3-6 cm	1 (100)	0	
> 6 cm	0	2 (40)	
Extranodal Tumour Extension			> 0.99
Absent	0	1 (20)	
Present	1 (100)	4 (80)	
pT Stage*			0.116
T3	4 (44.4)	1 (7.1)	
T4	5 (55.6)	12 (85.7)	
pN Stage*			> 0.99
N2	0	5 (50)	
N3	1 (100)	5 (50)	
pN Stage (8th Ed)			> 0.99
N1	0	5 (50)	
N3	1 (100)	5 (50)	

No of Sites with Distant Metastases			0.681
Single	4 (44.4)	8 (57.1)	
Multiple	5 (55.6)	6 (42.9)	
Adjuvant Treatment			> 0.99
Absent	5 (55.6)	8 (57.1)	
Present	4 (44.4)	6 (42.9)	
Type of Adjuvant therapy			> 0.99
Chemotherapy	4 (100)	4 (66.7)	
IFN	0	1 (16.7)	
Chemo & IFN	0	1 (16.7)	

* All cases not included
† Data not available for all cases
Abbreviations: ALM – Acral Lentiginous Melanoma, IFN – Interferon ($p < 0.05$ significant)

Table 25. Univariate analysis of clinicopathological parameters with overall metastases

Characteristics	**Overall Metastases**		**p value**
	Present (n=34)	**Absent (n=16)**	
Age (in years)			0.582
≤ 50	15 (44.1)	9 (56.3)	
51-60	10 (29.4)	5 (31.2)	
> 60	9 (26.5)	2 (12.5)	
Gender			> 0.99
Male	22 (64.7)	10 (62.5)	
Female	12 (35.3)	6 (37.5)	
Clinical Site			0.080
Cutaneous	24 (70.6)	15 (93.7)	
Anal	10 (29.4)	1 (6.3)	
Max Tumour Size			**0.032**
≤ 2 cm	11 (32.3)	11 (68.8)	
2-4 cm	16 (47.1)	2 (12.5)	
> 4 cm	7 (20.6)	3 (18.7)	
Invasive Subtype			0.510
SSM	2 (5.9)	0	
ALM	14 (41.2)	8 (50)	
Nodular	17 (50)	5 (31.3)	
Others	1 (2.9)	3 (18.7)	
Breslow Thickness (in mm)			0.163
≤ 4.00	6 (17.6)	6 (37.5)	
> 4.00	28 (82.4)	10 (62.5)	
Clark Level*			0.349
4	12 (36.4)	8 (53.3)	
5	21 (63.6)	7 (46.7)	
Growth Phase			> 0.99
Radial	2 (5.9)	0	
Vertical	32 (94.1)	16 (100)	
Ulceration			0.370
Absent	3 (8.8)	3 (18.7)	
Present	31 (91.2)	13 (81.3)	
Lymphovascular Invasion			0.514
Absent	9 (26.5)	6 (37.5)	
Present	25 (73.5)	10 (62.5)	
Perineural Invasion			> 0.99
Absent	20 (58.8)	9 (56.2)	
Present	14 (41.2)	7 (43.8)	

Tumour Infiltrating Lymphocytes			0.669
Absent	2 (5.9)	1 (6.3)	
Non brisk	23 (67.6)	13 (81.2)	
Brisk	9 (26.5)	2 (12.5)	
Regression			0.249
Absent	30 (88.2)	12 (75)	
Present	4 (11.8)	4 (25)	
Extent of Regression			> 0.99
0-25%	3 (75)	3 (75)	
25-50%	1 (25)	1 (25)	
Mitotic Index (per mm^2)			0.496
≤ 6	8 (23.5)	6 (37.5)	
6-12	8 (23.5)	4 (25)	
> 12	18 (53)	6 (37.5)	
Cellular Pigmentation			> 0.99
Absent	8 (23.5)	4 (25)	
Present	26 (76.5)	12 (75)	
Upward Scatter*			> 0.99
Absent	5 (15.2)	2 (13.3)	
Present	28 (84.8)	13 (86.7)	
Nest Formation*			0.406
Absent	7 (21.2)	1 (6.7)	
Present	26 (78.8)	14 (93.3)	
Lateral Circumscription*			0.530
Abrupt	1 (3.2)	1 (7.1)	
Continuous	30 (96.8)	13 (92.9)	
Cell Size			0.650
Medium	5 (14.7)	1 (6.3)	
Large	29 (85.3)	15 (93.7)	
Cell Shape			0.351
Epithelioid	20 (58.8)	12 (75)	
Spindled	14 (41.2)	4 (25)	
Tumour Giant Cells			0.650
Absent	29 (85.3)	15 (93.7)	
Present	5 (14.7)	1 (6.3)	
Insitu Peripheral Margin[†]			> 0.99
Involved/ < 1 mm	11 (47.8)	6 (54.5)	
1-10 mm	12 (52.2)	5 (45.5)	
Invasive Peripheral Margin[†]			0.396
Involved/ < 1 mm	5 (21.7)	5 (41.7)	
1-20 mm	14 (60.9)	5 (41.7)	
> 20 mm	4 (17.4)	2 (16.7)	
Invasive Deep Margin[†]			0.659
Involved/ < 1 mm	6 (46.2)	5 (62.5)	
≥ 1 mm	7 (53.8)	3 (37.5)	
pT Stage*			0.456
T3	6 (18.2)	4 (28.6)	
T4	27 (81.8)	10 (71.4)	
pN Stage*			> 0.99
N2	10 (50)	1 (100)	
N3	10 (50)	0	
pN Stage (8^{th} Ed)*			> 0.99
N1	12 (54.5)	1 (100)	
N3	10 (45.5)	0	
Adjuvant Treatment			0.074
Absent	23 (67.6)	15 (93.7)	
Present	11 (32.4)	1 (6.3)	
Type of Adjuvant therapy			> 0.99
Chemotherapy	8 (72.7)	1 (100)	

IFN	2 (18.2)	0
Chemo & IFN	1 (9.1)	0

* All cases not included
† Data not available for all cases
Abbreviations: SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, IFN – Interferon, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 26. Univariate analysis of clinicopathological parameters with onset of overall metastases

Characteristics	**Onset of Overall Metastases**		**p value**
	On presentation (n=21)	**On follow-up (n=13)**	
Age (in years)			0.363
≤ 50	11 (52.4)	4 (30.8)	
51-60	6 (28.6)	4 (30.8)	
> 60	4 (19)	5 (38.4)	
Gender			0.075
Male	11 (52.4)	11 (84.6)	
Female	10 (47.6)	2 (15.4)	
Clinical Site			0.704
Cutaneous	14 (66.7)	10 (76.9)	
Anal	7 (33.3)	3 (23.1)	
Max Tumour Size			0.223
≤ 2 cm	9 (42.9)	2 (15.4)	
2-4 cm	8 (38.1)	8 (61.5)	
> 4 cm	4 (19)	3 (23.1)	
Invasive Subtype			0.288
SSM	2 (9.5)	0	
ALM	7 (33.3)	7 (53.8)	
Nodular	12 (57.1)	5 (38.5)	
Others	0	1 (7.7)	
Breslow Thickness (in mm)			> 0.99
≤ 4.00	4 (19)	2 (15.4)	
> 4.00	17 (81)	11 (84.6)	
Clark Level*			0.067
4	5 (23.8)	7 (58.3)	
5	16 (76.2)	5 (41.7)	
Growth Phase			0.139
Radial	0	2 (15.4)	
Vertical	21 (100)	11 (84.6)	
Ulceration			0.544
Absent	1 (4.8)	2 (15.4)	
Present	20 (95.2)	11 (84.6)	
Lymphovascular Invasion			> 0.99
Absent	6 (28.6)	3 (23.1)	
Present	15 (71.4)	10 (76.9)	
Perineural Invasion			> 0.99
Absent	12 (57.1)	8 (61.5)	
Present	9 (42.9)	5 (38.5)	
Tumour Infiltrating Lymphocytes			0.282
Absent	2 (9.5)	0	
Non brisk	12 (57.1)	11 (84.6)	
Brisk	7 (33.3)	2 (15.4)	
Regression			0.627
Absent	19 (90.5)	11 (84.6)	
Present	2 (9.5)	2 (15.4)	
Extent of Regression			> 0.99
0-25%	1 (50)	2 (100)	

25-50%	1 (50)	0	
Mitotic Index (per mm^2)			> 0.99
≤ 6	5 (23.8)	3 (23.1)	
6-12	5 (23.8)	3 (23.1)	
> 12	11 (52.4)	7 (53.8)	
Cellular Pigmentation			0.444
Absent	6 (28.6)	2 (15.4)	
Present	15 (71.4)	11 (84.6)	
Upward Scatter*			0.625
Absent	4 (20)	1 (7.7)	
Present	16 (80)	12 (92.3)	
Nest Formation*			0.676
Absent	5 (25)	2 (15.4)	
Present	15 (75)	11 (84.6)	
Lateral Circumscription*			0.419
Abrupt	0	1 (7.7)	
Continuous	18 (100)	12 (92.3)	
Cell Size			0.132
Medium	5 (23.8)	0	
Large	16 (76.2)	13 (100)	
Cell Shape			0.728
Epithelioid	13 (61.9)	7 (53.8)	
Spindled	8 (38.1)	6 (46.2)	
Tumour Giant Cells			> 0.99
Absent	18 (85.7)	11 (84.6)	
Present	3 (14.3)	2 (15.4)	
Insitu Peripheral Margin[†]			0.220
Involved/ < 1 mm	7 (63.6)	4 (33.3)	
1-10 mm	4 (36.4)	8 (66.7)	
Invasive Peripheral Margin[†]			0.369
Involved/ < 1 mm	3 (27.3)	2 (16.7)	
1-20 mm	5 (45.5)	9 (75)	
> 20 mm	3 (27.3)	1 (8.3)	
Invasive Deep Margin[†]			0.266
Involved/ < 1 mm	5 (62.5)	1 (20)	
≥ 1 mm	3 (37.5)	4 (80)	
Clinical Satellite/In transit metastases			**0.030**
Absent	9 (42.9)	11 (84.6)	
Present	12 (57.1)	2 (15.4)	
Max Lymph Node Size			> 0.99
≤ 3 cm	1 (33.3)	4 (50)	
3-6 cm	1 (33.3)	3 (37.5)	
> 6 cm	1 (33.3)	1 (12.5)	
Extranodal Extension			> 0.99
Absent	1 (33.3)	4 (50)	
Present	3 (66.7)	4 (50)	
pT Stage*			0.379
T3	5 (23.8)	1 (8.3)	
T4	16 (76.2)	11 (91.7)	
pN Stage*			**0.020**
N2	9 (75)	1 (12.5)	
N3	3 (25)	7 (87.5)	
pN Stage (8th Ed)*			**0.027**
N1	10 (76.9)	2 (22.2)	
N3	3 (23.1)	7 (77.8)	
No of Sites with Distant Metastases			0.681
Single	8 (57.1)	4 (44.4)	
Multiple	6 (42.9)	5 (55.6)	

Adjuvant Treatment			> 0.99
Absent	14 (66.7)	9 (69.2)	
Present	7 (33.3)	4 (30.8)	
Type of Adjuvant therapy			0.109
Chemotherapy	6 (85.7)	2 (50)	
IFN	0	2 (50)	
Chemo & IFN	1 (14.3)	0	
Serum LDH Levels			> 0.99
Normal	2 (50)	3 (60)	
Elevated	2 (50)	2 (40)	

* All cases not included
† Data not available for all cases
Abbreviations: SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, IFN – Interferon, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 27. Univariate analysis of clinicopathological parameters with histological subtype of invasive melanoma

Characteristics	**Invasive Melanoma Subtype**		**p value**
	Acral lentiginous (n=22)	**Nodular (n=22)**	
Clinical Site			**< 0.001**
Cutaneous	22 (100)	11 (50)	
Anal	0	11 (50)	
Max Tumour Size			0.622
≤ 2 cm	11 (50)	10 (45.4)	
2-4 cm	8 (36.4)	6 (27.3)	
> 4 cm	3 (13.6)	6 (27.3)	
Breslow Thickness (in mm)			0.488
≤ 4.00	4 (18.2)	7 (31.8)	
> 4.00	18 (81.8)	15 (68.2)	
Clark Level*			> 0.99
4	8 (40)	8 (36.4)	
5	12 (60)	14 (63.6)	
Growth Phase			> 0.99
Radial	1 (4.5)	1 (4.5)	
Vertical	21 (95.5)	21 (95.5)	
Ulceration			0.664
Absent	4 (18.2)	2 (9.1)	
Present	18 (81.8)	20 (90.9)	
Lymphovascular Invasion			> 0.99
Absent	7 (31.8)	7 (31.8)	
Present	15 (68.2)	15 (68.2)	
Perineural Invasion			> 0.99
Absent	12 (54.5)	12 (54.5)	
Present	10 (45.5)	10 (45.5)	
Tumour Infiltrating Lymphocytes			0.856
Absent	1 (4.5)	1 (4.5)	
Non brisk	15 (68.2)	17 (77.3)	
Brisk	6 (27.3)	4 (18.2)	
Regression			0.095
Absent	16 (72.7)	21 (95.5)	
Present	6 (27.3)	1 (4.5)	
Extent of Regression			> 0.99
0-25%	4 (66.7)	1 (100)	
25-50%	2 (33.3)	0	

Mitotic Index (per mm^2)			0.925
≤ 6	6 (27.3)	6 (27.3)	
6-12	5 (22.7)	7 (31.8)	
> 12	11 (50)	9 (40.9)	
Cellular Pigmentation			0.457
Absent	6 (27.3)	3 (13.6)	
Present	16 (72.7)	19 (86.4)	
Upward Scatter*			0.087
Absent	1 (4.5)	5 (25)	
Present	21 (95.5)	15 (75)	
Nest Formation*			**0.003**
Absent	0	7 (35)	
Present	22 (100)	13 (65)	
Lateral Circumscription*			0.492
Abrupt	2 (9.1)	0	
Continuous	20 (90.9)	18 (100)	
Cell Size			> 0.99
Medium	2 (9.1)	3 (13.6)	
Large	20 (90.9)	19 (86.4)	
Cell Shape			0.203
Epithelioid	12 (54.5)	17 (77.3)	
Spindled	10 (45.5)	5 (22.7)	
Clinical Satellite/In transit metastases			0.510
Absent	14 (63.6)	17 (77.3)	
Present	8 (36.4)	5 (22.7)	
Max Lymph Node Size			0.286
≤ 3 cm	1 (20)	4 (80)	
3-6 cm	2 (40)	1 (20)	
> 6 cm	2 (40)	0	
Extranodal Tumour Extension			> 0.99
Absent	2 (40)	2 (40)	
Present	3 (60)	3 (60)	
pT Stage*			0.277
T3	3 (14.3)	6 (30)	
T4	18 (85.7)	14 (70)	
pN Stage*			0.650
N2	6 (54.5)	3 (37.5)	
N3	5 (45.5)	5 (62.5)	
pN Stage (8th Ed)*			0.650
N1	6 (54.5)	3 (37.5)	
N3	5 (45.5)	5 (62.5)	
cM Stage*			0.752
M1c	9 (42.9)	10 (52.6)	
M0	12 (57.1)	9 (47.4)	
No of Sites with Distant Metastases			0.680
Single	6 (60)	6 (46.2)	
Multiple	4 (40)	7 (53.8)	
Adjuvant Treatment			0.736
Absent	17 (77.3)	15 (68.2)	
Present	5 (22.7)	7 (31.8)	
Serum LDH Levels			**0.048**
Normal	1 (20)	4 (100)	
Elevated	4 (80)	0	

* All cases not included
Abbreviations: LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 28. Univariate analysis of clinicopathological characteristics for Overall Survival (OS) in patients with melanoma

Characteristics	Number (n=18)	Mean	95% CI	p value
Age (in years)				0.17
≤ 50	10	21.5	14.0, 29.1	
51-60	6	19.7	11.2, 28.1	
> 60	2	29.4	19.5, 39.3	
Gender				0.29
Male	11	25.2	18.5, 31.9	
Female	7	19.7	13.4, 25.9	
Clinical Site				0.28
Cutaneous	13	24.2	18.4, 30.0	
Anal	5	19.7	9.5, 29.8	
Max Tumour Size				**0.014**
≤ 2 cm	9	15.0	8.9, 21.2	
2-4 cm	7	28.7	21.5, 36.0	
> 4 cm	2	21.3	15.5, 27.2	
Invasive Subtype				0.51
ALM	8	23.0	16.1, 29.9	
Nodular	8	23.7	15.4, 31.9	
Breslow Thickness				0.28
≤ 4.00 mm	5	18.8	8.3, 29.4	
> 4.00 mm	13	24.1	18.2, 30.0	
Clark Level*				0.103
4	5	28.1	19.1, 37.2	
5	12	19.5	13.6, 25.5	
Growth phase				0.30
Radial	2	37.0	31.5, 42.5	
Vertical	16	21.8	16.6, 26.9	
Ulceration				0.91
Absent	4	24.7	11.6, 37.8	
Present	14	22.8	17.3, 28.2	
Lymphovascular Invasion				0.26
Absent	7	18.9	9.0, 28.7	
Present	11	24.7	18.9, 30.4	
Perineural Invasion				0.31
Absent	11	20.5	13.8, 27.1	
Present	7	26.1	18.8, 33.3	
Tumour Infiltrating Lymphocytes				0.09
Absent	2	11.0	6.2, 15.8	
Non brisk	13	24.9	18.8, 30.9	
Brisk	3	21.0	11.4, 30.6	
Regression				0.17
Absent	17	21.5	16.1, 26.6	
Present	1	32.3	20.8, 43.7	
Extent of Regression				---------
0-25%	1	32.3	20.8, 43.7	
25-50%	0			
Mitotic Index (per mm^2)				0.425
≤ 6	14	24	14.6, 33.4	
6-12	12	31.7	26.3, 37	
> 12	24	20.3	13.3, 27.2	
Cellular Pigmentation				0.64
Absent	4	16.8	13.2, 20.5	
Present	14	24.0	18.0, 30.0	

Upward Scatter*				**0.007**
Absent	2	8.5	7.8, 9.2	
Present	15	24.8	19.3, 30.2	
Nest Formation*				0.57
Absent	2	21.6	15.6, 27.6	
Present	15	23.8	18, 29.5	
Cell Size				**0.005**
Medium	4	12.0	9.6, 14.4	
Large	14	25.2	19.5, 30.8	
Cell Shape				0.97
Epithelioid	10	23.5	16.2, 30.8	
Spindled	8	22.3	15.3, 29.2	
Tumour Giant Cells				0.50
Absent	17	22.4	17.1, 27.6	
Present	1	21.5	12.2, 30.8	
Insitu Peripheral Margin[†]				0.62
Involved/ < 1 mm	7	23	15.6, 30.3	
1-10 mm	5	26.4	16.6, 36.2	
Invasive Peripheral Margin[†]				**0.04**
Involved/ < 1 mm	6	15.2	8.1, 22.3	
1-20 mm	5	29.9	21.8, 38	
> 20 mm	1	18.5	9.5, 27.5	
Invasive Deep Margin[†]				0.53
Involved/ < 1 mm	5	16	9.8, 22.2	
≥ 1 mm	3	21.3	10.6, 31.9	
Clinical Satellite/ In transit metastases				0.975
Absent	12	23.6	17.5, 29.7	
Present	6	21.3	13.7, 28.9	
Max Lymph Node Size				0.33
≤ 3 cm	2	33.0	21.9, 44.1	
3-6 cm	0			
> 6 cm	1	20.5	14.3, 26.7	
Extranodal Tumour Extension				0.92
Absent	1	25.0	25, 25	
Present	2	32.7	19.3, 46.0	
pT Stage*				0.173
T3	4	16.6	5.6, 27.6	
T4	13	24.1	18.2, 30	
pN Stage*				**0.018**
N2	3	15.3	13.9, 16.7	
N3	3	31.7	22, 41.4	
pN Stage (8th Ed)*				**0.008**
N1	4	15	13.8, 16.2	
N3	3	31.7	22, 41.4	
No of Sites with Distant Metastases				0.824
Single	4	27.2	16, 38.4	
Multiple	5	26	17, 35	
Adjuvant Treatment				0.63
Absent	12	21.7	15.6, 27.8	
Present	6	25.5	16.7, 34.3	
Serum LDH Levels				0.28
Normal	1	41.0	41, 41	
Elevated	2	20.3	15.6, 24.9	
Recurrence				0.492
Absent	7	27.2	20.7, 33.8	

Present	2	32.3	18.5, 46.2	
Metastases				0.154
Absent	9	17.6	12, 23.2	
Present	9	26.8	19.7, 33.8	

* All cases not included
† Data not available for all cases
Abbreviations: CI – Confidence Interval, SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 29. Univariate analysis of clinicopathological characteristics for Distant Metastases Free Survival (DMFS) in patients with melanoma

Characteristics	**Number (n=14)**	**Mean**	**95% CI**	**p value**
Age (in years)				0.70
≤ 50	6	25.0	16.6, 33.5	
51-60	3	16.0	3.5, 28.5	
> 60	5	20.4	8.4, 32.4	
Gender				0.81
Male	11	22.9	15.7, 30.0	
Female	3	15.9	7.3, 24.6	
Clinical Site				0.31
Cutaneous	12	20.0	12.6, 27.5	
Anal	2	25.6	17.9, 33.3	
Max Tumour Size				0.87
≤ 2 cm	3	18.8	11.2, 26.5	
2-4 cm	9	22.2	13.7, 30.6	
> 4 cm	2	20.8	13.5, 28.0	
Invasive Subtype				0.274
ALM	9	16.1	8.1, 24.1	
Nodular	5	25.0	16.7, 33.2	
Breslow Thickness				0.40
≤ 4.00 mm	2	29.0	11.4, 46.6	
> 4.00 mm	12	20.9	14.5, 27.4	
Clark Level*				0.726
4	6	25	15.8, 34.1	
5	7	21.7	13.1, 30.2	
Growth phase				0.71
Radial	2	21.5	1.4, 41.6	
Vertical	12	22.1	15.3, 29.0	
Ulceration				0.53
Absent	3	18.3	2.4, 34.1	
Present	11	23.0	16.0, 30.0	
Lymphovascular Invasion				0.12
Absent	5	15.5	1.9, 29.1	
Present	9	24.4	17.2, 31.6	
Perineural Invasion				0.23
Absent	8	17.1	9.6, 24.5	
Present	6	26.2	16.9, 35.4	
Tumour Infiltrating Lymphocytes				0.19
Absent	1	8.0	8, 8	
Non brisk	10	25.1	18.2, 32.0	
Brisk	3	8.4	5.3, 11.5	
Regression				0.48
Absent	12	21.5	14.4, 28.6	
Present	2	25.1	12.4, 37.8	

Extent of Regression				NA
0-25%	2	25.1	12.4, 37.8	
25-50%	0			
Mitotic Index (per mm^2)				0.647
≤ 6	14	25	13.5, 36.5	
6-12	12	25.6	17.9, 33.3	
> 12	24	17.9	9.5, 26.2	
Cellular Pigmentation				0.78
Absent	3	18.4	10.8, 26.0	
Present	11	22.3	14.9, 29.8	
Upward Scatter				--------
Absent	0			
Present	14	21.2	14.7, 27.7	
Nest Formation				0.76
Absent	2	10.8	7.1, 14.4	
Present	12	21.5	14.4, 28.6	
Cell Size				0.75
Medium	1	10.8	6.9, 14.7	
Large	13	21.4	14.7, 28.1	
Cell Shape				0.46
Epithelioid	7	25.9	17.3, 34.5	
Spindled	7	18.4	10.4, 26.4	
Tumour Giant Cells				0.17
Absent	11	23.4	16.6, 30.2	
Present	3	11.6	1.4, 21.8	
Insitu Peripheral Margin[†]				0.86
Involved/ < 1 mm	5	18.6	8.1, 29	
1-10 mm	7	21.8	11.9, 31.8	
Invasive Peripheral Margin[†]				0.34
Involved/ < 1 mm	2	6.7	6.1, 7.2	
1-20 mm	8	22.7	13.6, 31.7	
> 20 mm	2	21.2	14.3, 28	
Invasive Deep Margin[†]				0.93
Involved/ < 1 mm	2	14.3	4.2, 24.5	
≥ 1 mm	3	23.5	7.3, 39.7	
Clinical Satellite/In transit metastases				**0.006**
Absent	7	26.9	19.7, 34.1	
Present	7	11.6	1.9, 21.4	
Max Lymph Node Size				0.73
≤ 3 cm	3	22.3	8.8, 35.7	
3-6 cm	0			
> 6 cm	2	16.5	4.7, 28.3	
Extranodal Tumour Extension				0.60
Absent	1	25.0	25, 25	
Present	4	19.4	7.5, 31.3	
pT Stage*				0.124
T3	1	40	40, 40	
T4	12	20.8	14.4, 27.3	
pN Stage*				**0.008**
N2	5	5.7	4, 7.4	
N3	5	21	11.8, 30.2	
pN Stage (8^{th} Ed)*				**0.011**
N1	5	5.9	4.3, 7.5	
N3	5	21	11.8, 30.2	

No of Sites with Distant Metastases				**0.038**
Single	8	9.7	2.3, 17	
Multiple	6	24.8	16.5, 33.2	
Adjuvant Treatment				0.75
Absent	8	23.4	15.1, 31.7	
Present	6	22.0	13.0, 30.9	
Serum LDH Levels				0.65
Normal	4	15.3	3.5, 27.0	
Elevated	4	15.3	7.1, 23.4	

* All cases not included
† Data not available for all cases
Abbreviations: CI – Confidence Interval, SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 30. Univariate analysis of clinicopathological characteristics for Recurrence Free Survival (RFS) in patients with melanoma

Characteristics	Number (n=5)	Mean	95% CI	p value
Age (in years)				0.35
≤ 50	4	25.2	16.0, 34.3	
51-60	1	19.0	3.8, 34.3	
> 60	0			
Gender				0.31
Male	3	32.2	24.7, 39.8	
Female	2	23.5	17.2, 29.8	
Clinical Site				0.25
Cutaneous	2	34.6	27.7, 41.6	
Anal	3	21.1	13.0, 29.2	
Max Tumour Size				> 0.99
≤ 2 cm	1	27.0	27, 27	
2-4 cm	3	30.7	21.7, 39.6	
> 4 cm	1	20.7	13.7, 27.6	
Invasive Subtype				0.53
ALM	1	36.6	30.3, 42.9	
Nodular	4	20.8	14.0, 27.6	
Breslow Thickness				---------
≤ 4.00 mm	0			
> 4.00 mm	5	27.6	19.9, 35.3	
Clark Level				0.454
4	1	34	23.5, 44.5	
5	4	27.2	18.4, 35.9	
Growth phase				0.10
Radial	1	10.0	10, 10	
Vertical	4	31.2	24.0, 38.4	
Ulceration				0.30
Absent	1	10.0	10, 10	
Present	4	30.9	23.6, 38.3	
Lymphovascular Invasion				0.64
Absent	1	11.5	9.4, 13.6	
Present	4	30.5	22.9, 38.1	
Perineural Invasion				0.71
Absent	2	20.0	14.2, 25.8	
Present	3	30.9	22.4, 39.3	

Tumour Infiltrating Lymphocytes				---------
Absent	0			
Non brisk	5	28.3	20.4, 36.3	
Brisk	0			
Regression				---------
Absent	5	26.9	18.3, 35.4	
Present	0			
Mitotic Index (per mm^2)				0.97
≤ 6	14	29.3	13.9, 44.8	
6-12	12	24.4	17.2, 31.6	
> 12	24	32.5	23.2, 41.7	
Cellular Pigmentation				0.81
Absent	1	21.8	16.2, 27.3	
Present	4	29.7	21.6, 37.9	
Upward Scatter				---------
Absent	0			
Present	5	29.2	21.7, 36.8	
Nest Formation				0.82
Absent	1	13	11.6, 14.4	
Present	4	30.1	22.2, 38	
Cell Size				---------
Medium	0			
Large	5	28.8	21.1, 36.5	
Cell Shape				0.72
Epithelioid	3	27.4	17.0, 37.8	
Spindled	2	32.5	24.3, 40.6	
Tumour Giant Cells				0.87
Absent	4	30.9	23.4, 38.5	
Present	1	27.0	27, 27	
Insitu Peripheral Margin[†]				0.78
Involved/ < 1 mm	2	28.5	16.6, 40.5	
1-10 mm	2	31.1	20.8, 41.5	
Invasive Peripheral Margin[†]				0.10
Involved/ < 1 mm	1	6.7	6.1, 7.2	
1-20 mm	3	30	20.7, 39.3	
> 20 mm	0			
Invasive Deep Margin[†]				0.74
Involved/ < 1 mm	2	20.7	10.5, 30.8	
≥ 1 mm	2	24.5	9.2, 39.8	
Clinical Satellite/In transit metastases				0.095
Absent	3	32.1	24.6, 39.5	
Present	2	10.6	8.2, 13.0	
Max Lymph Node Size				---------
≤ 3 cm	3	12.8	7.9, 17.6	
3-6 cm	0			
> 6 cm	0			
Extranodal Tumour Extension				0.98
Absent	1	18.5	9.5, 27.5	
Present	2	16.2	11.0, 21.4	
pT Stage				---------
T3	0			
T4	5	27.6	19.9, 35.3	
pN Stage*				0.381
N2	1	7.5	6.6, 8.4	
N3	3	18.6	13, 24.1	

pN Stage (8th Ed)*				0.381
N1	1	7.5	6.6, 8.4	
N3	3	18.6	13, 24.1	
No of Sites with Distant Metastases				0.907
Single	1	17.3	11.5, 23.2	
Multiple	3	28.9	19, 38.7	
Adjuvant Treatment				0.52
Absent	2	32.5	23.2, 41.7	
Present	3	22.0	16.2, 27.8	
Serum LDH Levels				0.54
Normal	2	13.6	7.1, 20.2	
Elevated	1	20.3	12.2, 28.3	

* All cases not included

† Data not available for all cases

Abbreviations: CI – Confidence Interval, SSM – Superficial Spreading Melanoma, ALM – Acral Lentiginous Melanoma, LDH – Lactate Dehydrogenase ($p < 0.05$ significant)

Table 31. Analysis of clinicopathological characteristics with metastases based on Breslow thickness

		Breslow Thickness							
		≤ 4mm				> 4mm			
		Metastases				Metastases			
		Present	Absent	RR (95% CI)	p-value	Present	Absent	RR (95% CI)	p-value
Age (in years)	≤ 50	2	4	0.66 (0.16, 2.66)	0.40	10	8	1.38 (0.70, 2.73)	0.52
	>50	3	3			8	12		
Gender	Male	3	5	0.75 (0.19, 2.83)	0.57	13	11	1.52 (0.69, 3.35)	0.33
	Female	2	2			5	9		
Clinical Site	Anal	2	1	2.0 (0.58, 6.79)	0.522	7	1	2.39 (1.39, 4.09)	**0.016**
	Cutaneous	3	6			11	19		
Max Tumour Size	≤ 2 cm	4	5	1.33 (0.23, 7.74)	> 0.99	6	7	0.96 (0.47, 1.96)	> 0.99
	> 2 cm	1	2			12	13		
Invasive Subtype*	ALM	2	2	1.17 (0.32, 4.28)	> 0.99	8	10	0.67 (0.35, 1.25)	0.296
	Nodular	3	4			10	5		
Growth Phase	Radial	1	0	2.75 (1.25, 6.00)	0.416	1	0	2.17 (1.53, 3.09)	0.47
	Vertical	4	7			17	20		
Ulceration	Absent	1	1	1.25 (0.25, 6.07)	> 0.99	2	2	1.06 (0.37, 3.01)	> 0.99
	Present	4	6			16	18		
Lymphovascular Invasion	Absent	2	3	0.93 (0.23, 3.68)	> 0.99	6	4	1.4 (0.72, 2.72)	0.47
	Present	3	4			12	16		
Perineural Invasion	Absent	4	5	1.33 (0.23, 7.74)	> 0.99	10	10	1.12 (0.57, 2.21)	0.76
	Present	1	2			8	10		
Clinical Satellite/ ITM	Present	0	0	NA	> 0.99	8	6	1.37 (0.72, 2.64)	0.50
	Absent	5	7			10	14		
TIL	Absent	0	1	NA	> 0.99	1	1	1.06 (0.25, 4.42)	> 0.99
	Present	5	6			17	19		
Regression	Absent	5	5	NA	0.46	15	17	0.94 (0.39, 2.26)	> 0.99
	Present	0	2			3	3		
Extent of Regression	0-25%	0	0	NA	> 0.99	2	2	1.0 (0.18, 5.46)	> 0.99
	25-50%	0	2			1	1		
Clark Level*	4	2	3	1.0 (0.21, 4.56)	> 0.99	5	10	0.59 (0.26, 1.31)	0.198
	5	2	3			13	10		
Cellular Pigmentation	Absent	1	2	0.75 (0.13, 4.35)	> 0.99	5	4	1.24 (0.61, 2.52)	0.71
	Present	4	5			13	16		
Cell size	Medium	0	2	NA	0.46	4	0	2.43 (1.62, 3.63)	**0.04**
	Large	5	5			14	20		
Cell shape	Epithelioid	4	6	0.8 (0.16, 3.88)	> 0.99	10	12	0.91 (0.46, 1.78)	> 0.99
	Spindled	1	1			8	8		
Max Lymph Node Size	≤ 3 cm	0	0	NA		3	2	1.2 (0.41, 3.51)	> 0.99
	>3 cm	0	0			3	3		
Extranodal Extension	Absent	0	0	NA		1	4	0.24 (0.04, 1.44)	0.08
	Present	0	0			5	1		
pT Stage*	T3	0	0	NA		1	0	2.17 (1.53, 3.09)	0.47
	T4	4	5			17	20		
pN Stage*	N2	0	0	NA		5	6	0.76 (0.33, 1.72)	0.67
	N3	0	0			6	4		
pN Stage (8th Ed)*	N1	0	1	NA		5	7	0.69 (0.30, 1.61)	0.67
	N3	0	0			6	4		
Serum LDH	Normal	0	0	NA		4	1	0.8 (0.52, 1.24)	> 0.99
	Elevated	0	0			4	0		

* All cases not included

Abbreviations: ALM – Acral Lentiginous Melanoma, ITM – In transit metastases, TIL – Tumour Infiltrating Lymphocytes, LDH – Lactate Dehydrogenase, NA – Not applicable ($p < 0.05$ significant)

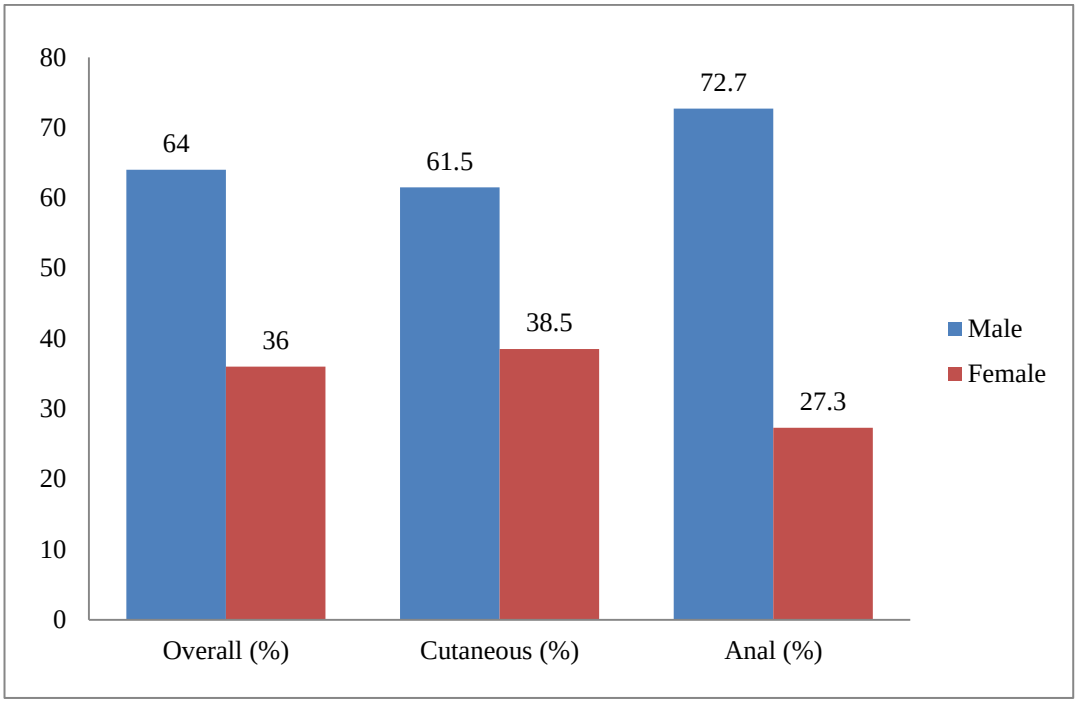

Figure 56. Gender distribution in melanoma

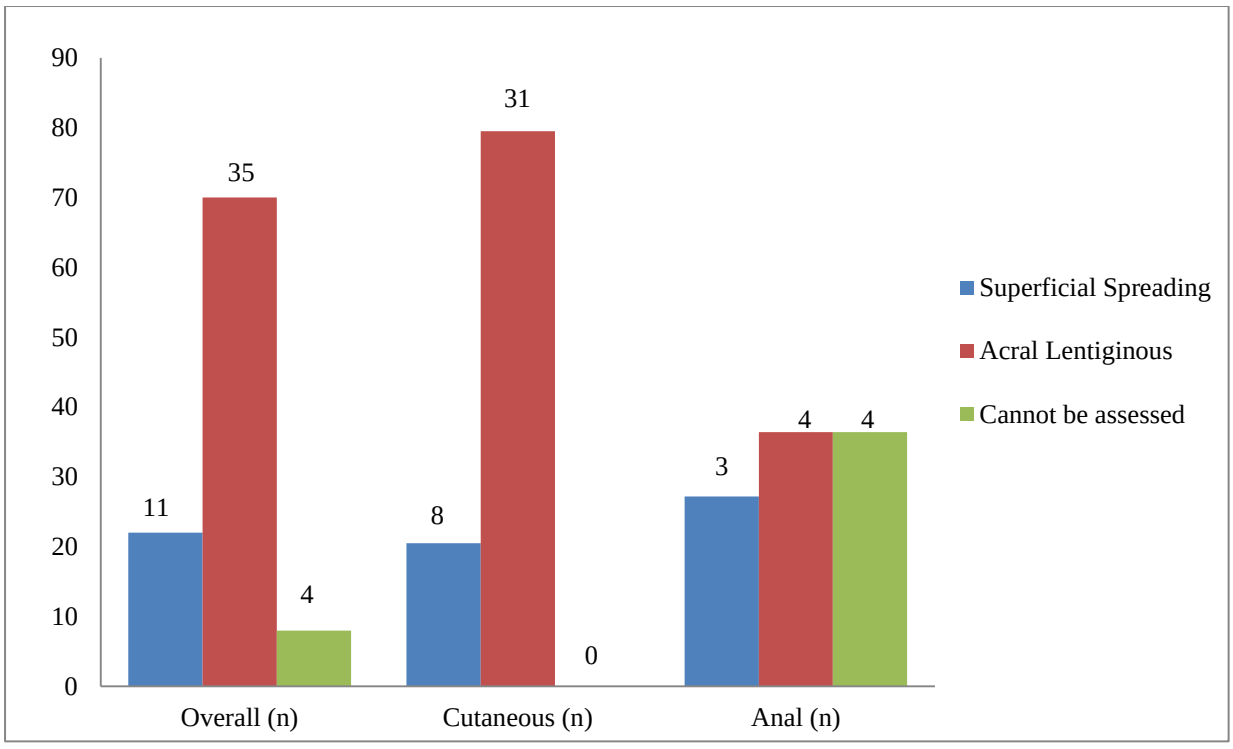

Figure 57. Histological subtypes of in situ component in melanoma

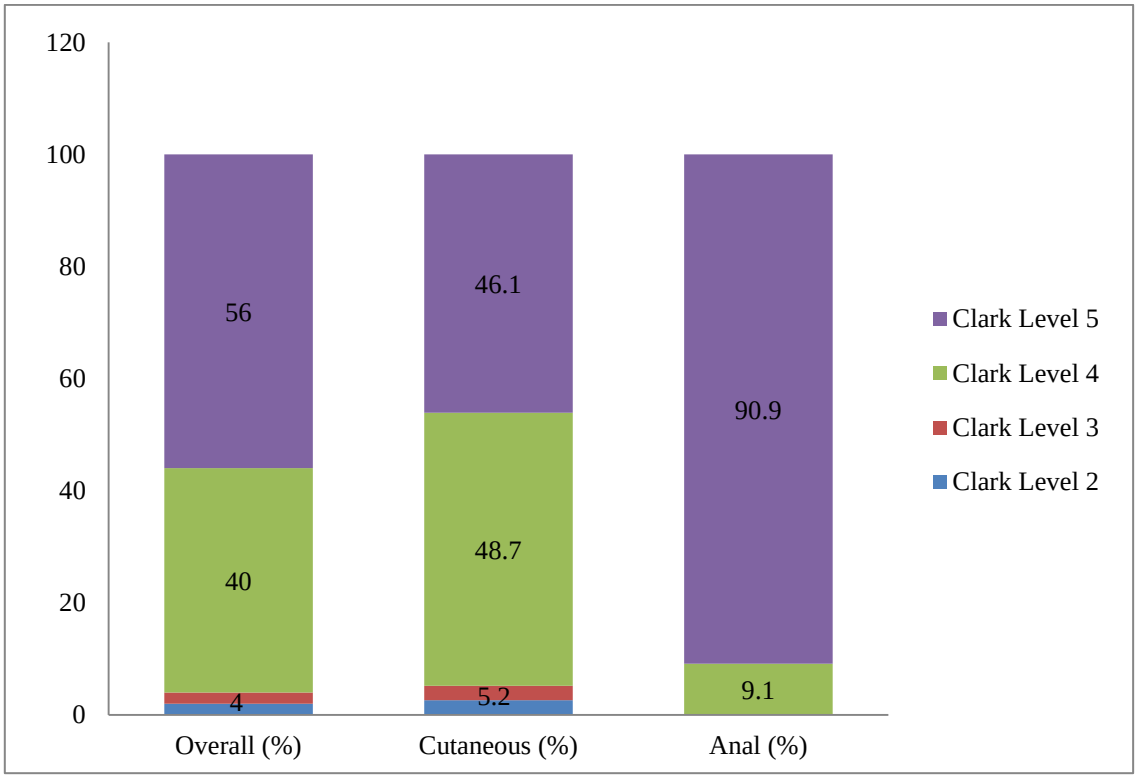

Figure 58. Distribution of Clark levels of invasion in melanoma

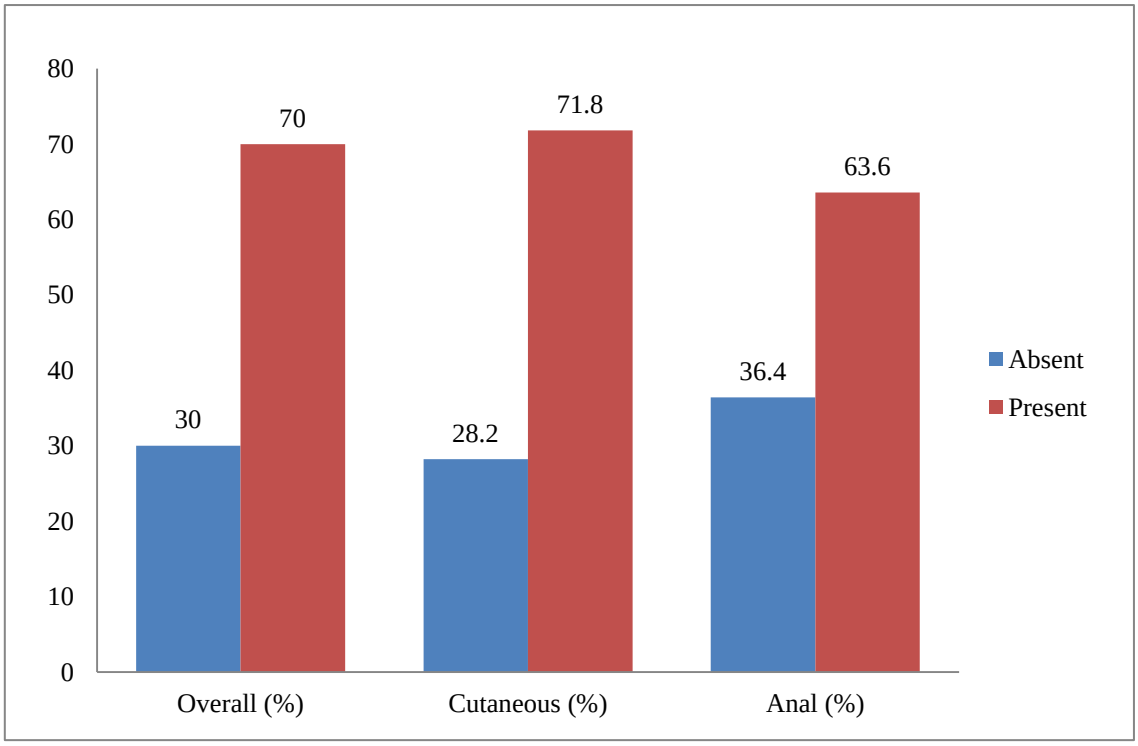

Figure 59. Lymphovascular invasion in melanoma

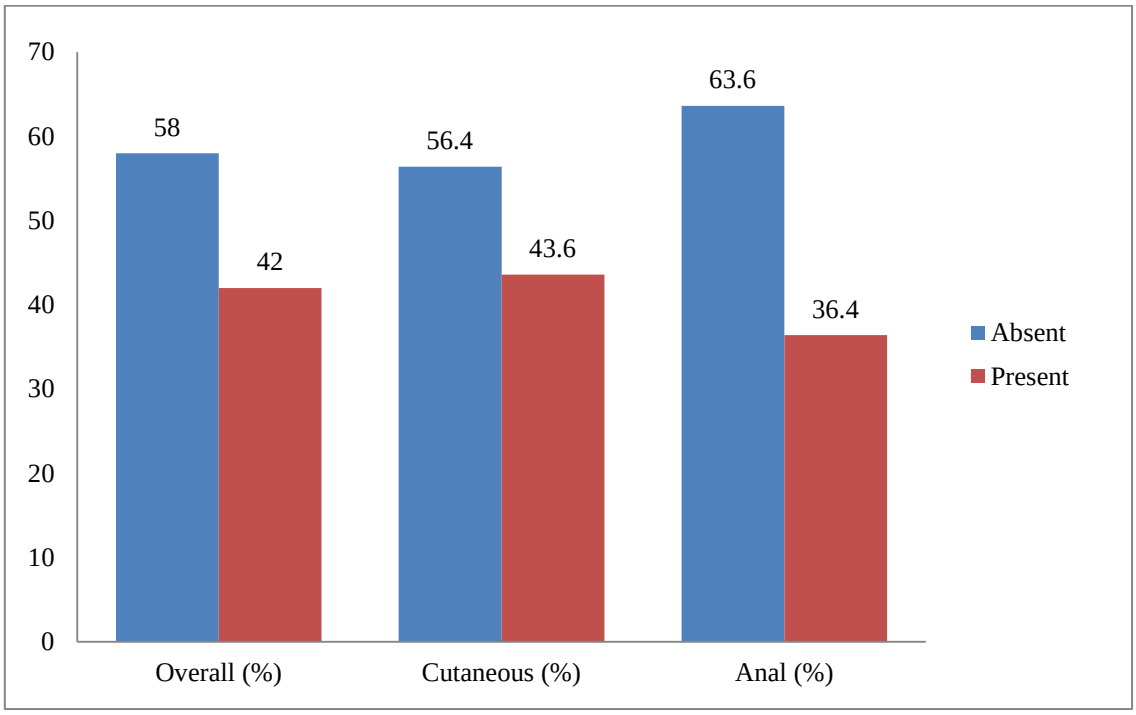

Figure 60. Perineural invasion in melanoma

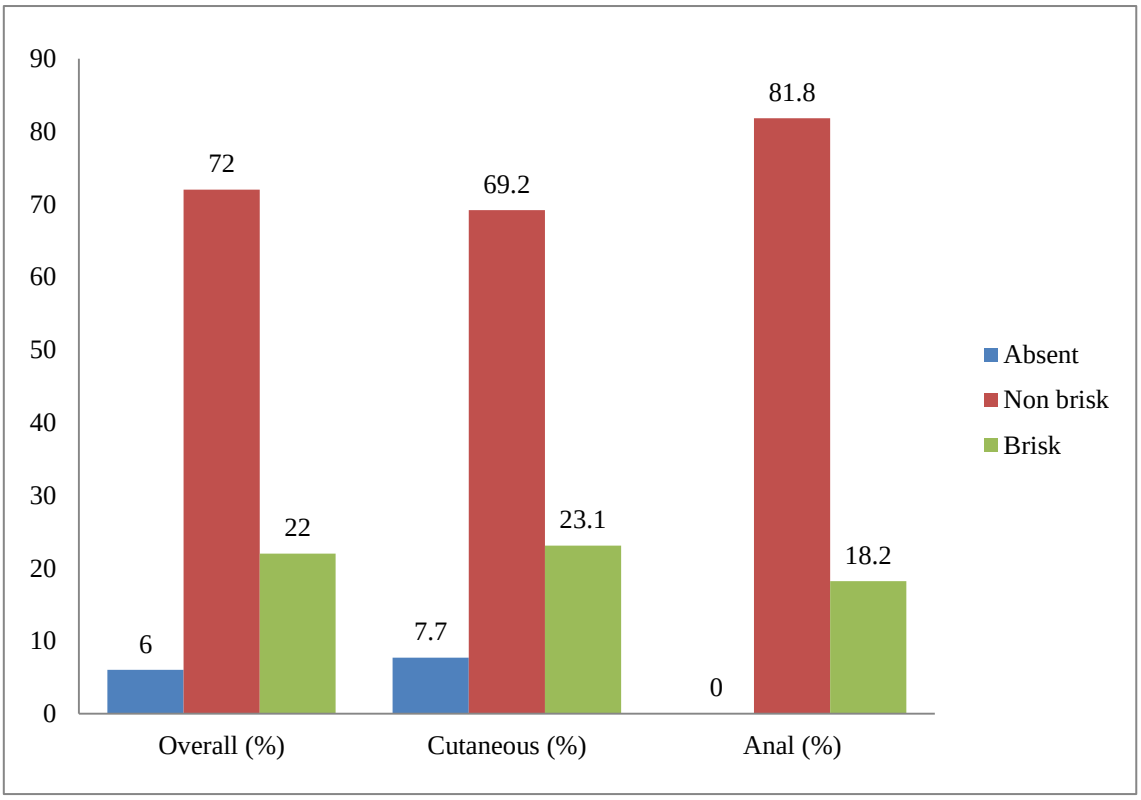

Figure 61. Response of tumour infiltrating lymphocytes (TIL) in melanoma

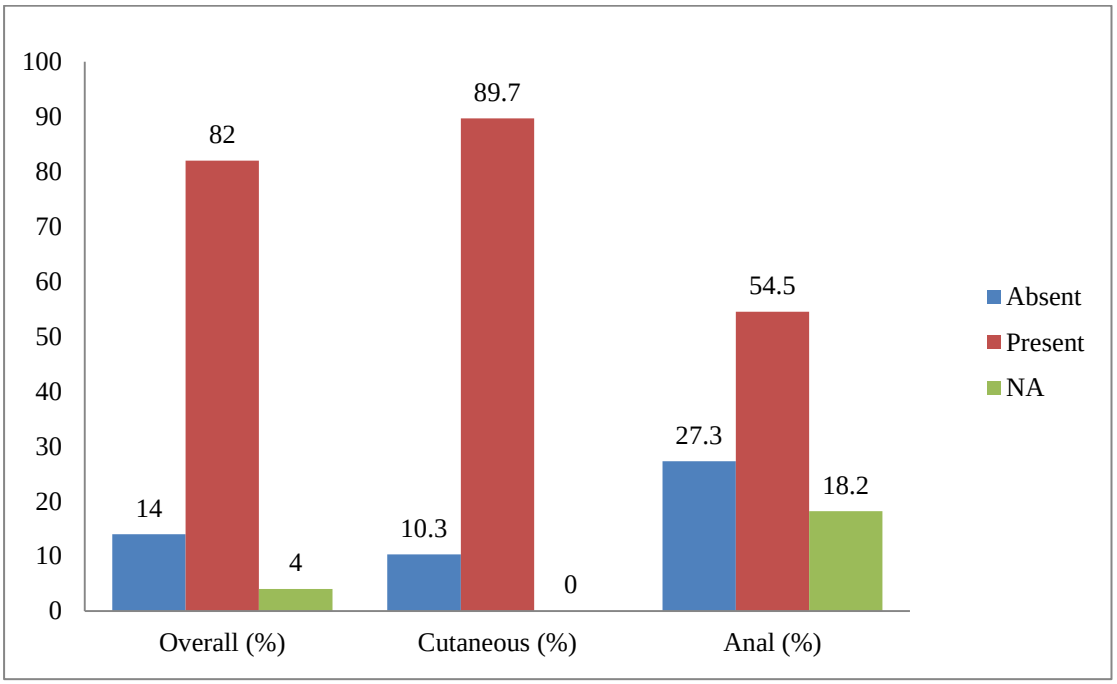

Figure 62. Upward scatter of intra-epidermal melanocytes in melanoma
(NA-Not Applicable)

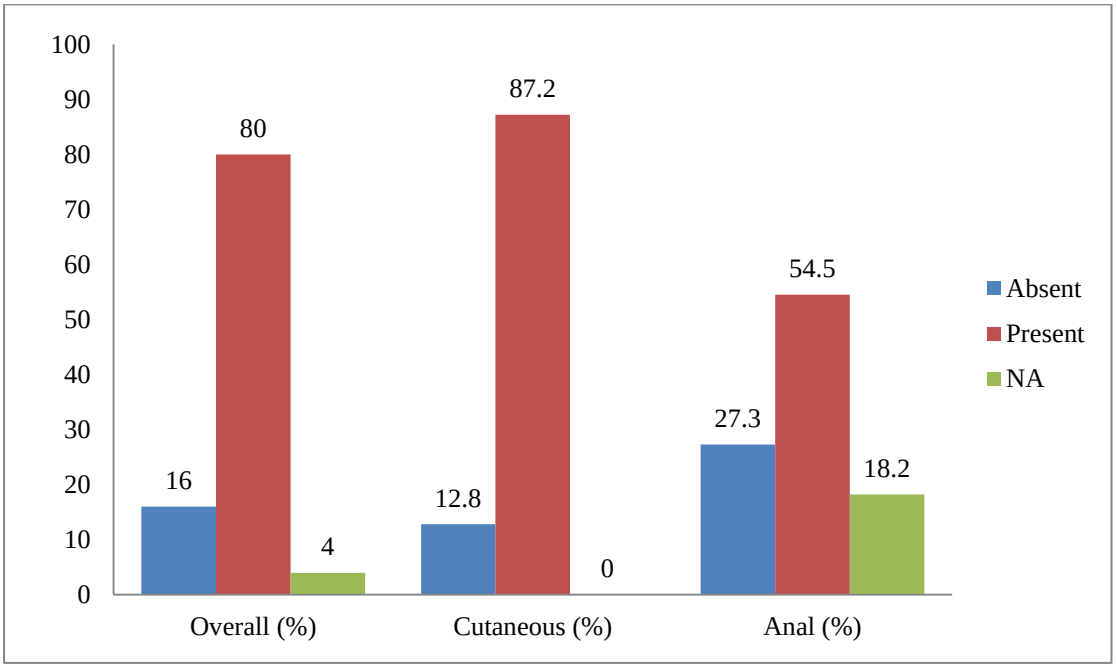

Figure 63. Nest formation of intra-epidermal melanocytes in melanoma
(NA-Not Applicable)

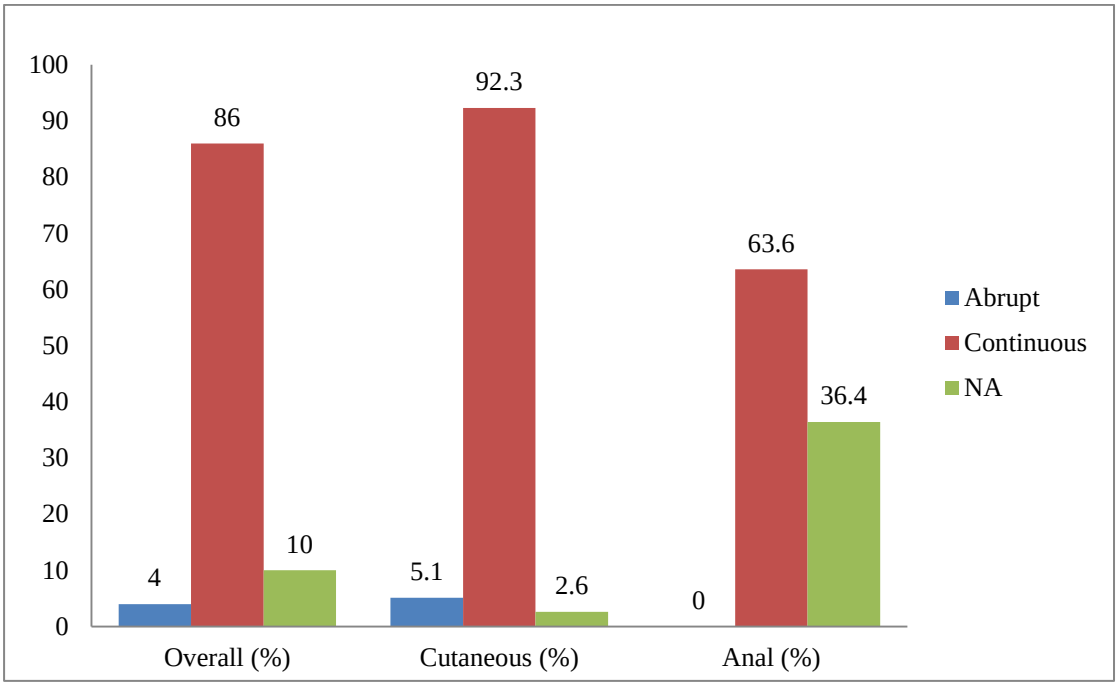

Figure 64. Lateral circumscription of intra-epidermal melanocytes in melanoma
(NA-Not Applicable)

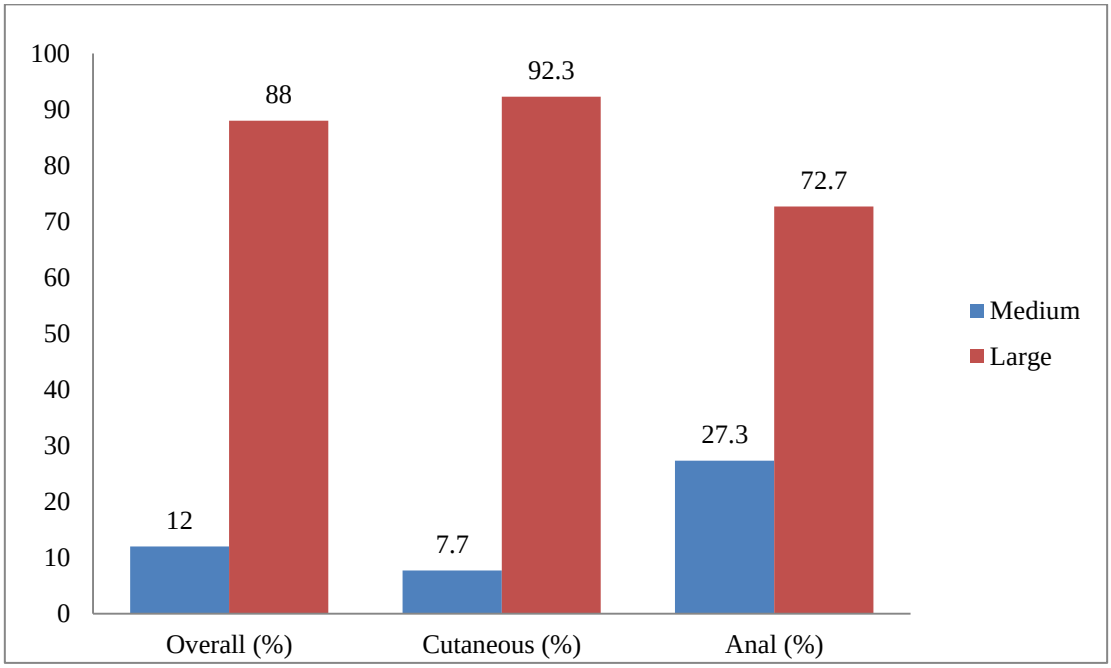

Figure 65. Cell size of atypical melanocytes in melanoma

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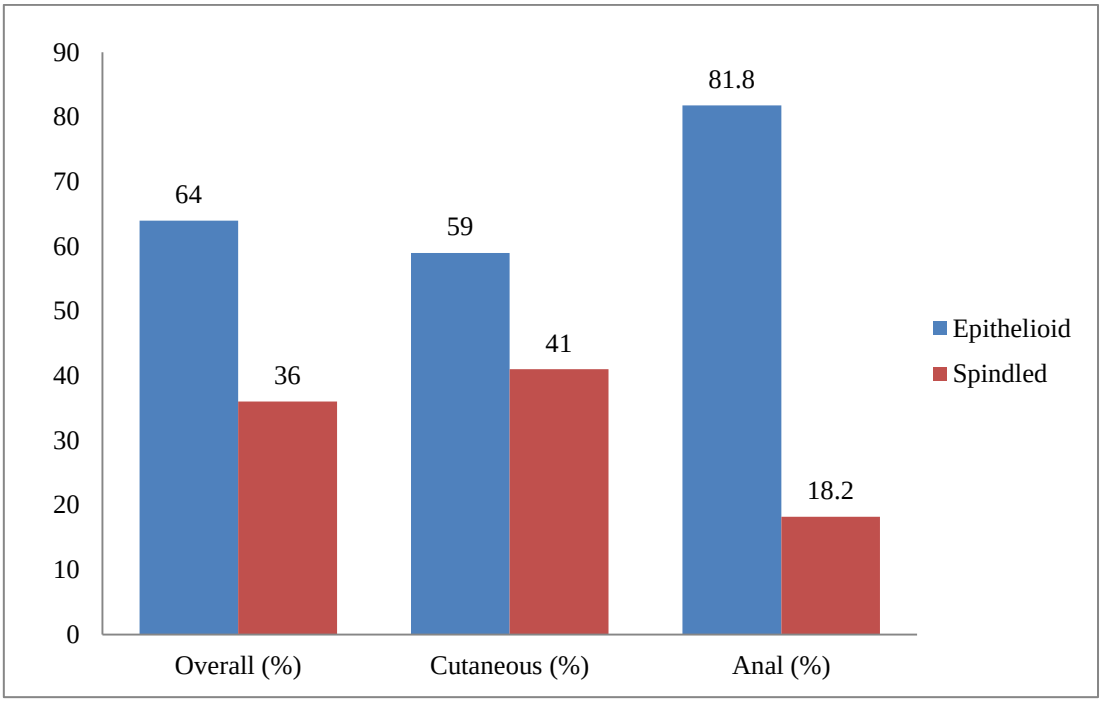

Figure 66. Cell shape of atypical melanocytes in melanoma

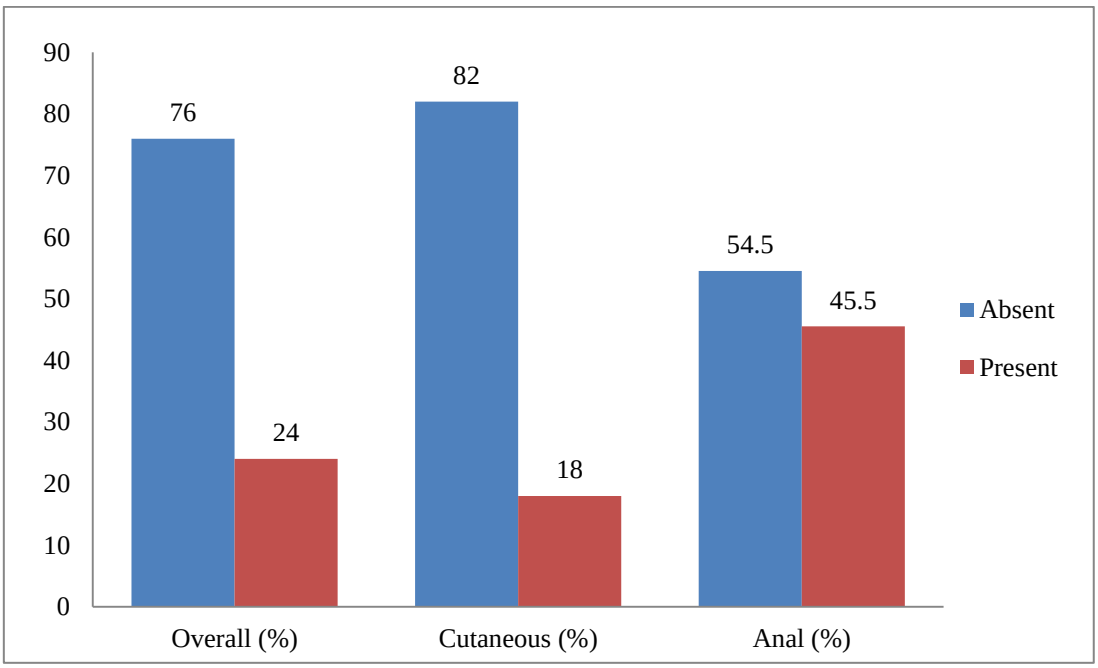

Figure 67. Adjuvant treatment in melanoma

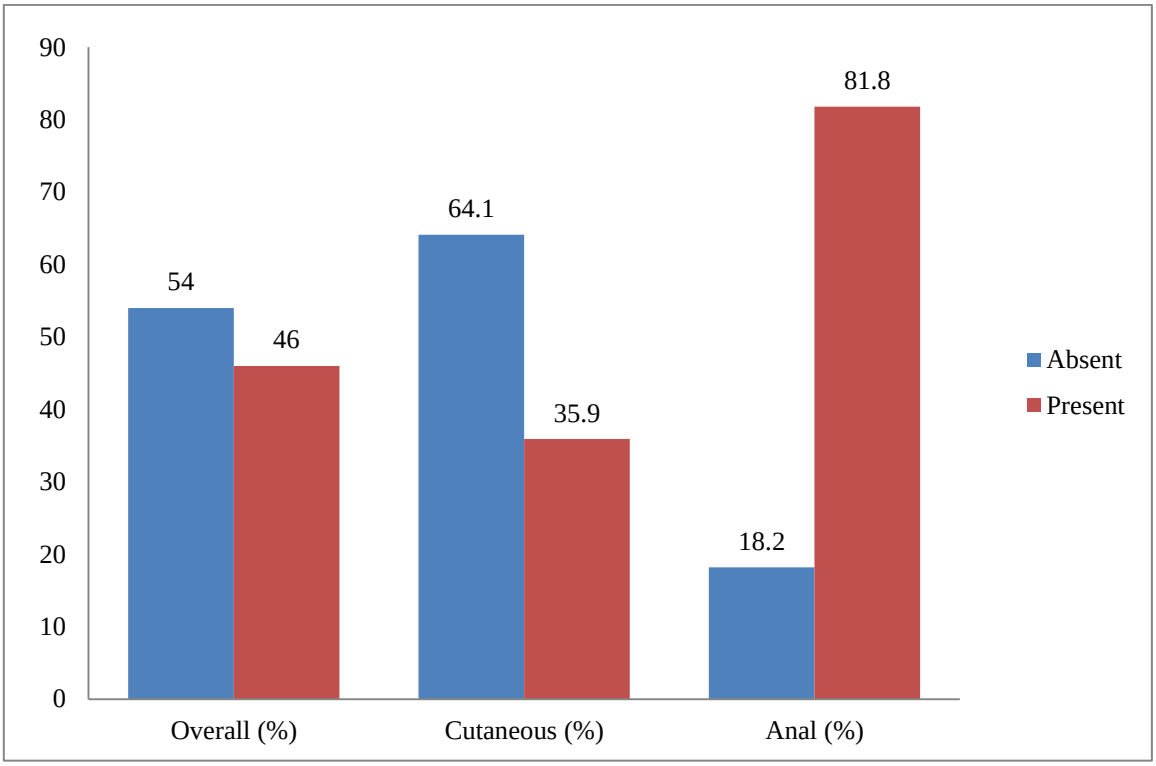

Figure 68. Frequency of metastases in melanoma

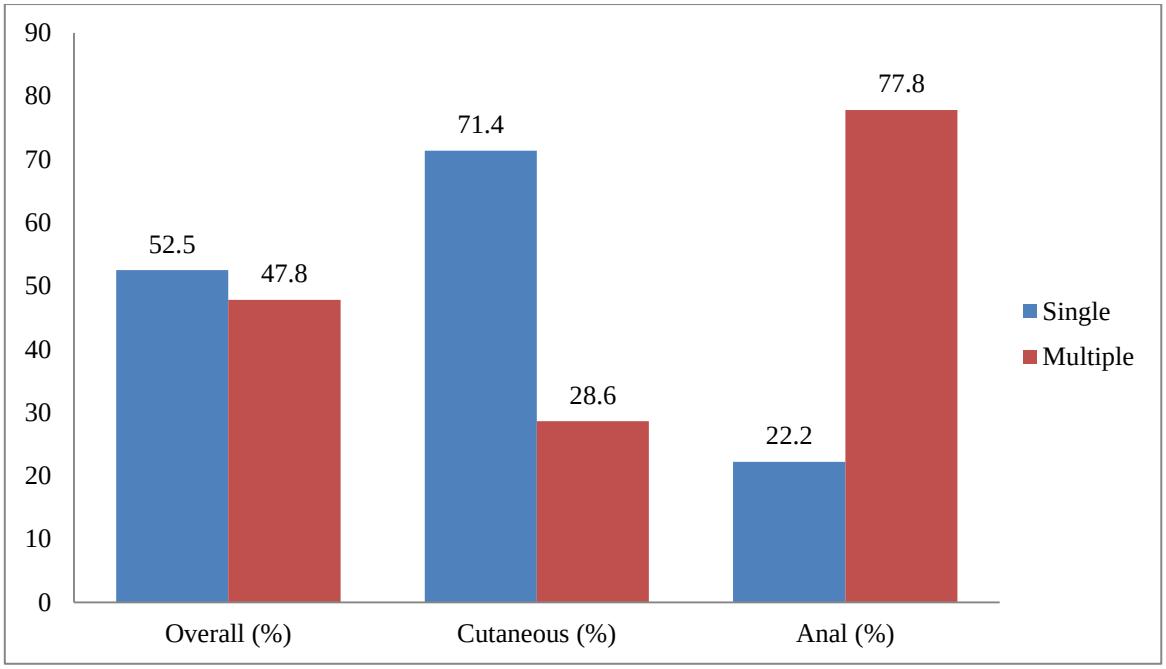

Figure 69. Distribution by number of sites of distant metastases in melanoma

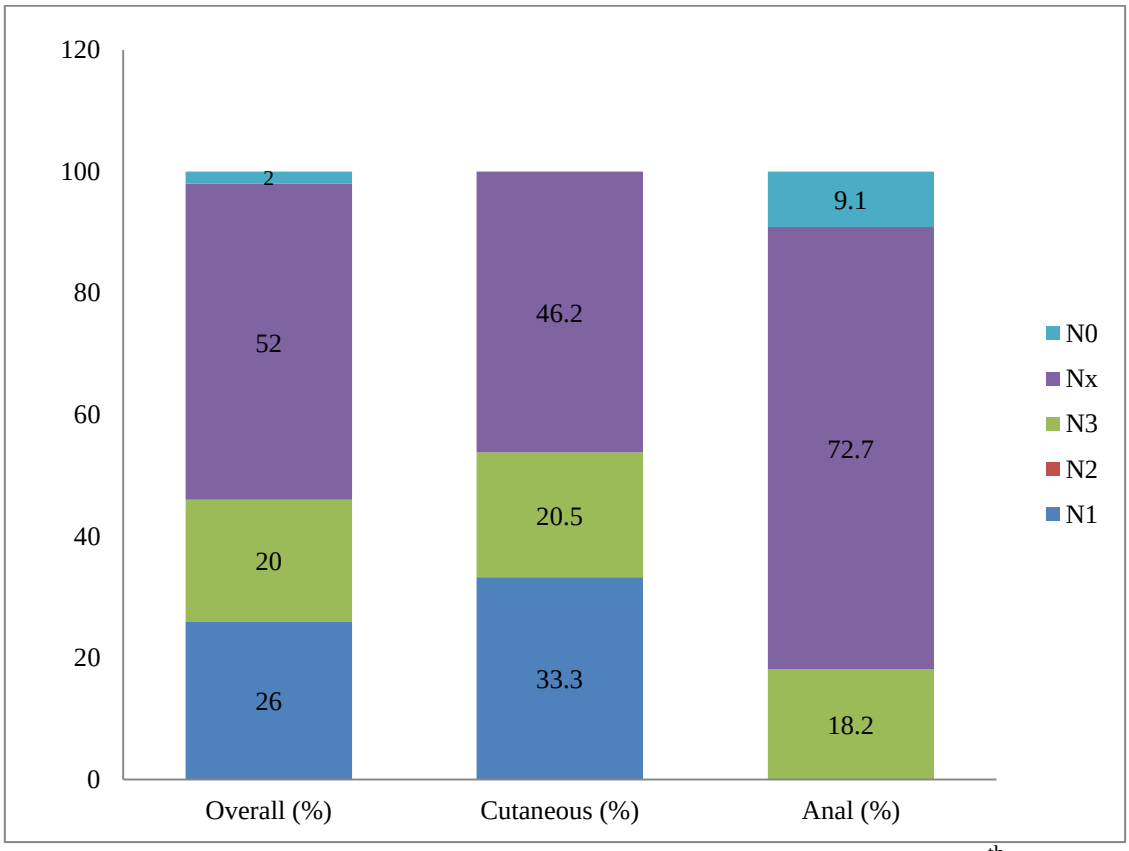

Figure 70. Distribution by pathological N (pN) stage in melanoma – AJCC 8th edition classification

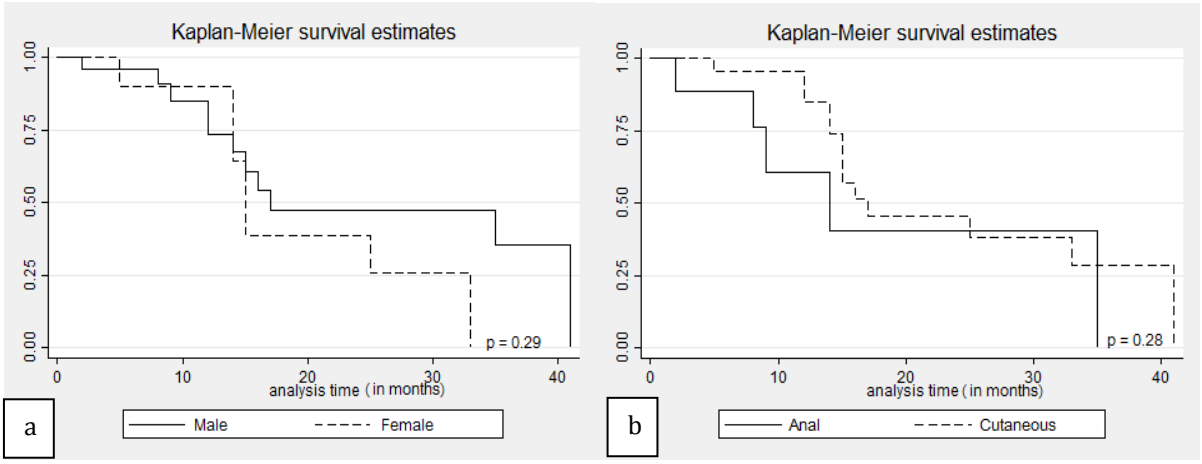

Figure 71. Overall Survival (OS) in melanoma patients with respect to gender(a) and clinical site(b) ($p < 0.05$ significant)

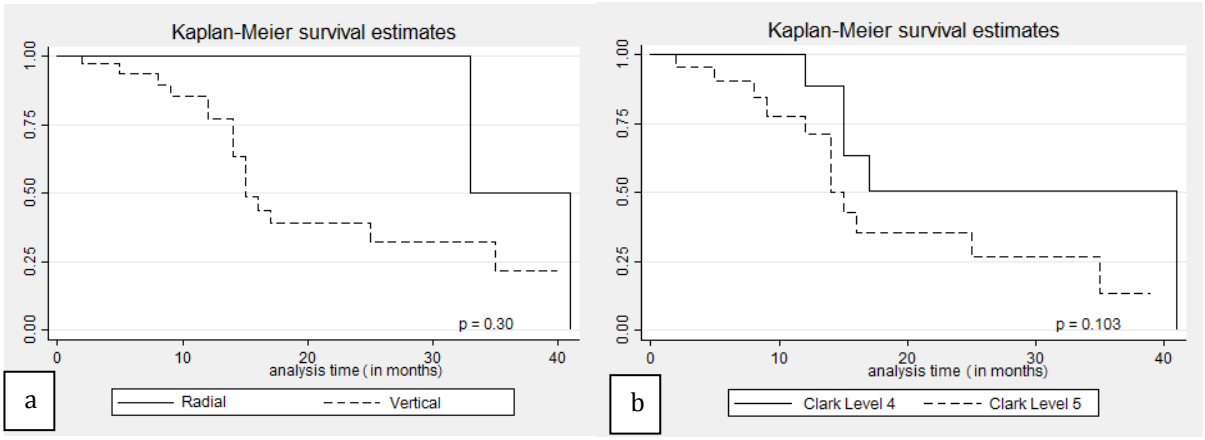

Figure 72. Overall Survival (OS) in melanoma patients with respect to growth phase(a) and Clark level(b) ($p < 0.05$ significant)

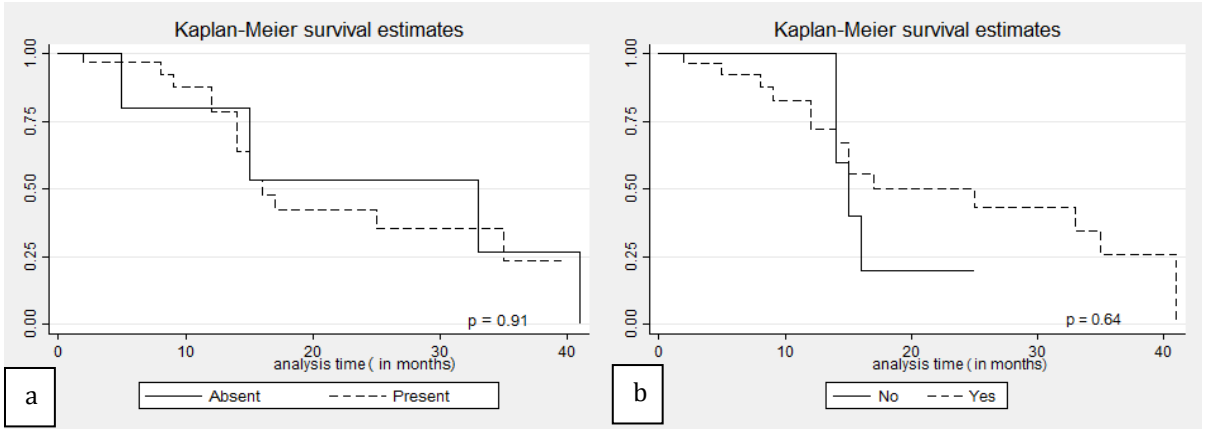

Figure 73. Overall Survival (OS) in melanoma patients with respect to ulceration(a) and cellular pigmentation(b) ($p < 0.05$ significant)

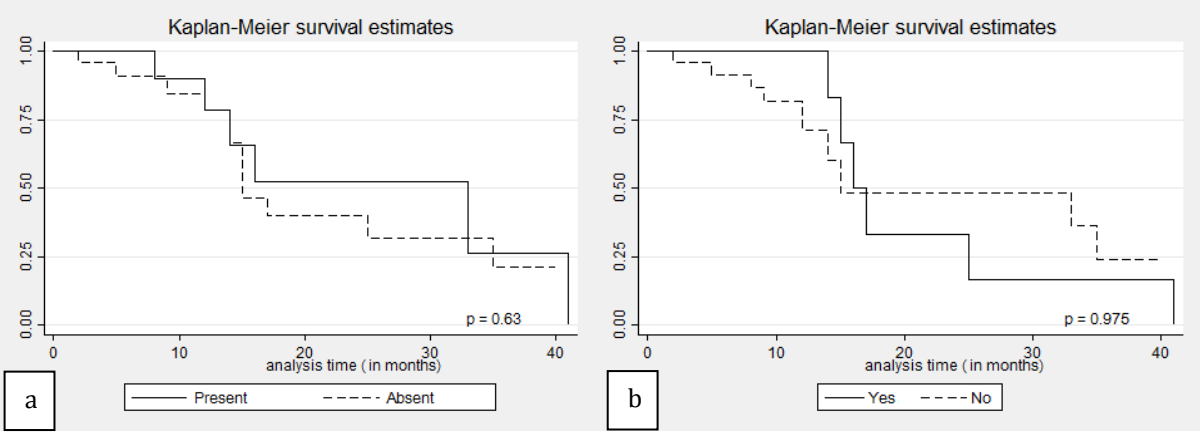

Figure 74. Overall Survival (OS) in melanoma patients with respect to adjuvant treatment(a) and clinical satellite/ in transit metastases(b) ($p < 0.05$ significant)

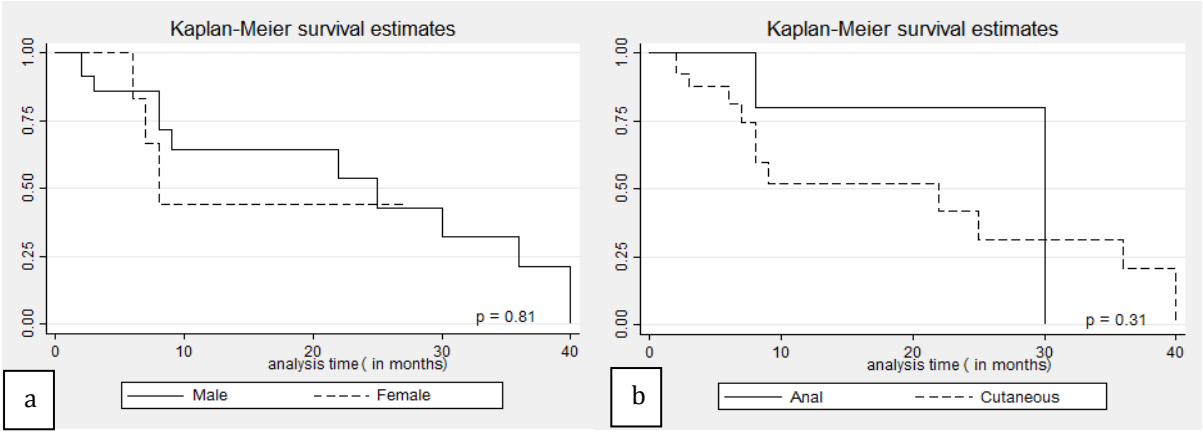

Figure 75. Distant Metastases Free Survival (DMFS) in melanoma patients with respect to gender(a) and clinical site(b) ($p < 0.05$ significant)

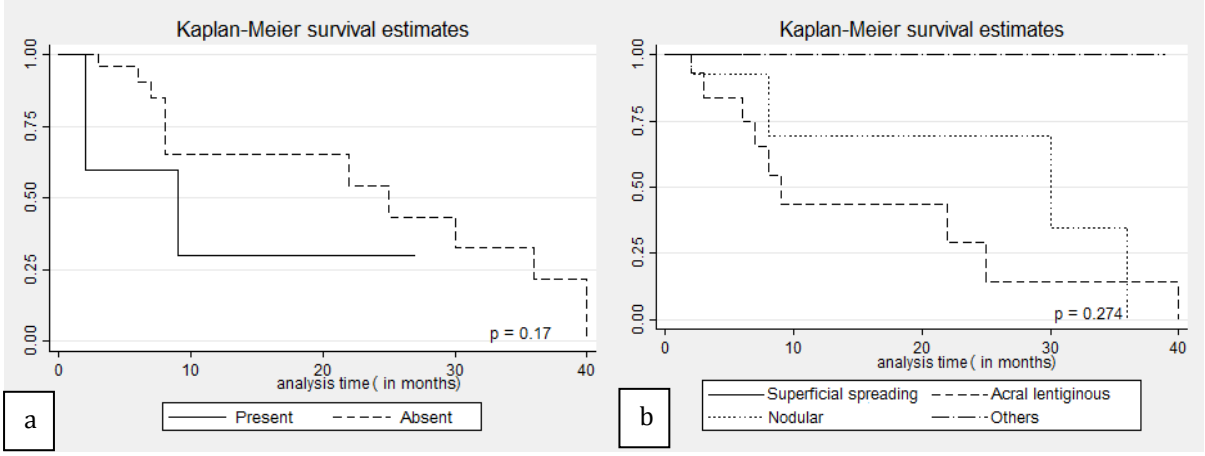

Figure 76. Distant Metastases Free Survival (DMFS) in melanoma patients with respect to histological subtype of invasive melanoma(a) and tumour giant cells(b) ($p < 0.05$ significant)

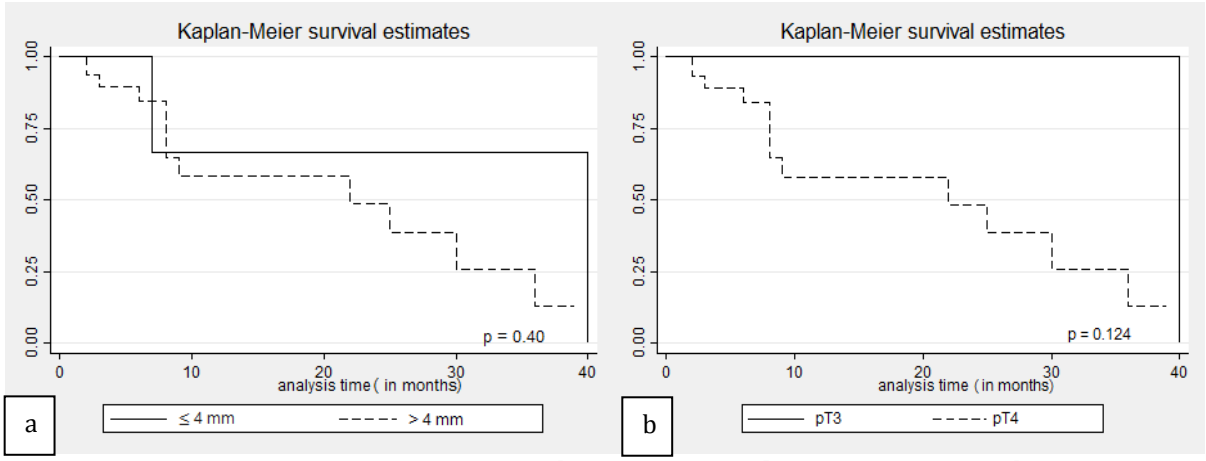

Figure 77. Distant Metastases Free Survival (DMFS) in melanoma patients with respect to Breslow thickness(a) and pT stage(b) ($p < 0.05$ significant)

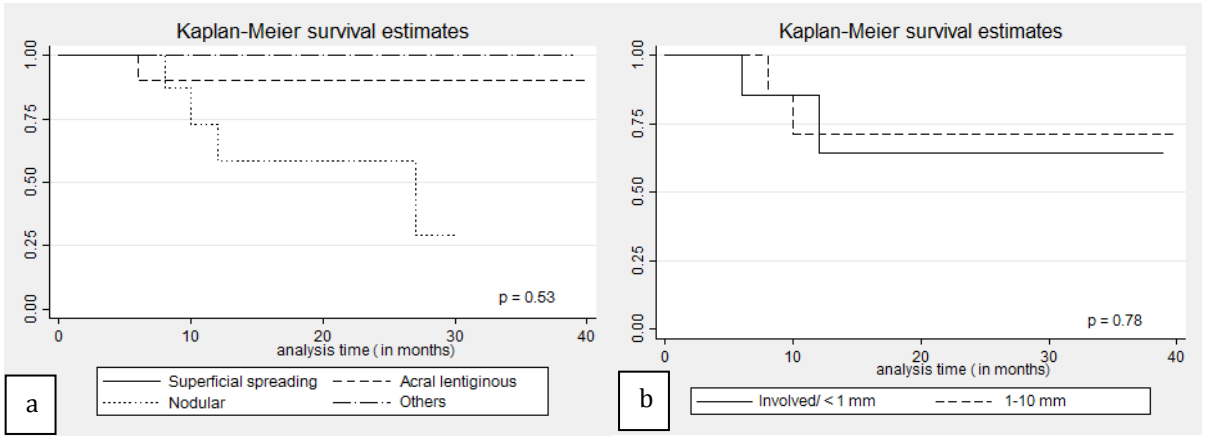

Figure 78. Recurrence Free Survival (RFS) in melanoma patients with respect to histological subtype of invasive melanoma(a) and peripheral margin of in situ component(b) ($p < 0.05$ significant)

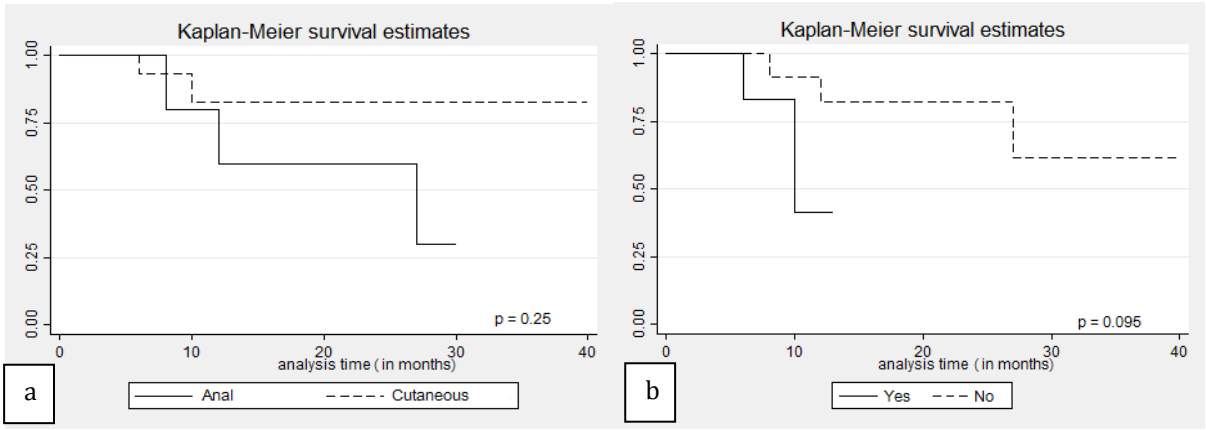

Figure 79. Recurrence Free Survival (RFS) in melanoma patients with respect to clinical site(a) and clinical satellite/ in transit metastases(b) ($p < 0.05$ significant)

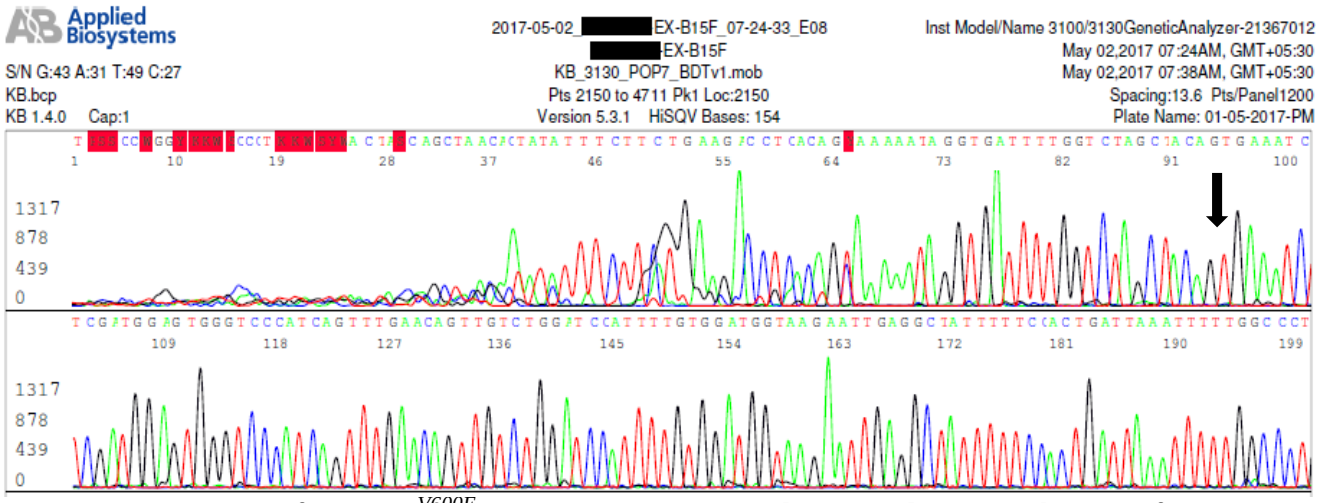

Figure 80. Sequencing for *BRAF*^{V600E} mutation using Forward primer, depicting absence of mutation - *BRAF wild type* (indicated by arrow)

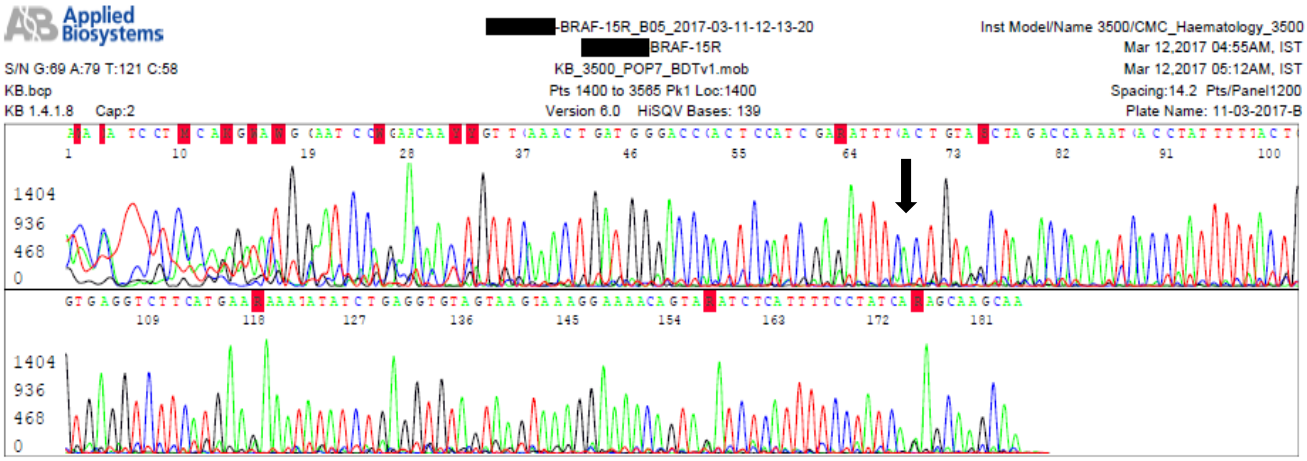

Figure 81. Sequencing for *BRAF*^{V600E} mutation using Reverse primer, depicting absence of mutation - *BRAF wild type* (indicated by arrow)

4. DATA LABELS

serno	biopno	hospno	age	gender	residence	clinsite	spectype	size	maxtumour	insitu	histotype	regres	regpres	permarg	pmarni	deepmarg
1	3529/14	789929F	45	2	Tamilnadu	Right leg	1	3x3	3	3						
2	4421/14	795946F	47	1	West Bengal	Anal canal	2	1x1x0.9	1	2	1	1		5		5
3	14393/14	821956F	47	2	Tamilnadu	Left heel	3	3x2.5x2	3	2	2	1		1		5
4	26958/14	003937G	28	1	West Bengal	Left foot	1	1x1x0.3	1	2	2	1		3	3	5
5	34677/14	288721F	57	2	Tamilnadu	Left great toe	4	2.8x2.6x0.6	2.8	2	1	1		3	28	5
6	5775/15	159373G	42	2	West Bengal	Right foot	1	1x0.7x0.5	1	2	2	1		2		5
7	14304/15	949063F	48	2	Tamilnadu	Anal canal	6	NA		3						
8	40661/14	079184G	55	1	Jharkand	Anal canal	2	1.3x0.7x0.2	1.3	1						
9	24212/13	622270F	20	2	West Bengal	Anal canal	2	1x1	1	2	1	1		5		5
10	27326/13	643116F	52	1	Orissa	Left foot	1	3x3	3	2	1	1		5		5
11	38717/13	689905F	48	1	Andhra Pradesh	Anal canal	2	1x0.8x0.2	1	3						
12	43144/13	981366D	55	1	Bihar	Anal canal	5	3x2.6x1	3	2	2	1		5		5
13	44788/15	345125G	57	1	West Bengal	Anal canal	5	0.6x0.5x0.1	0.6	1						
14	23946/13	617996F	56	1	Tamilnadu	Left toe	1	0.5x0.5	0.5	2	2	1		5		5
15	3454/15	147061G	56	1	Kerala	Right foot	1	1.5x1.1x0.2	1.5	2	2	1		1		5
16	5694/14	797788F	40	2	Tamilnadu	Right sole	1	5x5	5	2	2	2	1	3	4	5
17	37510/13	702435F	50	1	Tamilnadu	Anal canal	2	1x0.5x0.2	1	2	2	1		1		5
18	49921/15	992050F	60	1	Tamilnadu	Right heel	1	0.8x0.8	0.8	2	2	1		5		5
19	6773/13	416113F	40	1	West Bengal	Right fourth toe	4	4x3.5x2.9	4	2	2	2	1	1		5
20	1267/15	066515G	40	1	Jharkand	Left great toe	4	2.2x2x2	2.2	2	2	1		1		5
21	26114/14	000758G	51	2	West Bengal	Right foot	1	1x0.4x0.3	1	2	2	1		5		5
22	50349/15	374762D	87	1	Tamilnadu	Left great toe	1	1x0.5x0.5	1	2	2	1		3	2	5
23	10352/13	432991F	33	2	Assam	Right foot	1	1.5x0.6x0.6	1.5	2	2	1		1		5
24	47866/14	932544F	114	1	Tamilnadu	Right little toe	4	3.1x1.3x0.8	3.1	2	1	1		1		5
25	46629/15	367851G	50	1	Jharkand	Left great toe	4	5x4x2	5	2	2	2	2	3	13	5
26	26353/13	634589F	75	1	West Bengal	Left little toe	1	0.8x0.3	0.8	2	2	1		3	5	5
27	32256/13	670148F	40	1	West Bengal	Right heel	1	4x4	4	2	2	1		5		5
28	30834/13	666509F	43	1	West Bengal	Right foot	6	NA		2	2	1		3	2	5
29	9685/13	223233F	46	1	West Bengal	Anal canal	2	1.5x1.5	1.5	1						
30	7364/13	399738F	57	1	West Bengal	Left foot	1	3x2	3	2	2	1		3	7	5
31	38766/13	706227F	65	1	Jharkand	Left great toe	4	3x2.5x1.1	3	2	2	2	1	3	13	5
32	39153/15	322980G	62	1	Chattisgarh	Right foot	1	1.8x1x0.5	1.8	2	2	2	1	3	3	5
33	5834/13	407777F	57	2	West Bengal	Vulva	1	1x0.5	1	2	2	1		1		5
34	45715/14	100258G	45	2	West Bengal	Anal canal	5	0.4x0.4	0.4	3						
35	8781/14	032314L	49	2	Tamilnadu	Left heel	1	1.1x0.4x0.3	1.1	2	2	1		5		5
36	31890/15	0000001	56	2	NA	Left foot	1	1.5x1x0.2	1.5	2	2	1		3	5	5
37	923/13	0000002	60	1	NA	Right great toe	1	2x1.5x0.1	2	2	2	1		1		5
38	23573/13	0000003	75	2	NA	Right foot	1	8x1	8	2	1	1		5		5
39	22543/13	0000004	47	1	NA	Right forearm	5	1x0.8x0.2	1	2	1	1		1		5
40	20517/14	0000005	70	2	NA	Left heel	1	2x1x0.5	2	2	2	1		5		5
41	29655/14	022539G	41	1	West Bengal	Left thumb	1	1.5x0.5x0.5	1.5	2	2	1		3	3	5
42	587/15	131428G	74	1	Bangladesh	Left middle finger	4	3.7x3.5x1.5	3.7	2	1	1		3	10	5
43	26012/15	228577G	86	1	Tamilnadu	Right little finger	4	2.9x2.5x1.1	2.9	2	2	1		1		5
44	3412/13	397591F	36	1	West Bengal	Left index finger	1	3x1x0.7	3	2	2	1		5		5

invasive	histotype1	subtype	breslow	ulcer	mitoses	lvi	stltrans	stmpres	pni	growth	til	regression	rgrpres	clark	clarkno	inspmarg	inspmni	invpmarg	invpmni	invdmarg	invdmni
2	4			1	1	2	1		1	2	1	1		4		5		5		5	
2	4		3.5	2	18	1	1		1	2	2	1		2	5	5		5		5	
2	2		25	2	30	2	2	Left thigh	2	2	2	1		2	5	1		1		3	2
2	4		7.25	2	16	1	1		1	2	1	1		2	5	3	3	3	2	1	
2	4		5.53	2	3	2	1		2	2	3	1		2	5	3	28	3	28	5	
2	2		5.2	1	16	1	1		2	2	1	1		2	5	2		2		1	
2	4		14.08	2	30	2	1		2	2	2	1		2	5	5		5		5	
2	4		6.2	2	22	1	1		1	2	2	1		2	5	5		5		5	
2	4		3.78	2	1	2	1		1	2	2	1		2	5	5		5		5	
2	1		3.13	2	3	2	1		1	2	1	1		2	5	5		5		5	
2	4		4.75	2	3	1	1		1	2	3	1		2	5	5		5		5	
2	4		17.68	2	12	2	1		2	2	2	1		2	5	5		5		1	
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2	2		3.5	2	19	1	1		1	2	3	2	1	2	5	3	4	3	4	3	1
2	4		7.8	2	74	2	1		1	2	2	1		2	5	1		1		1	
2	2		5.03	2	6	2	1		1	2	2	1		2	5	5		5		5	
2	6	Pigment synthesising	29	2	3	2	1		2	2	2	2	1	2	5	1		3	5	5	
2	4		22	2	45	2	2	Left leg	2	2	2	1		2	5	1		3	30	5	
2	4		3.38	2	3	1	1		1	2	2	1		2	5	5		5		5	
2	2		5.3	2	1	1	1		1	2	2	1		2	4	3	2	3	2	5	
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2	2		20	2	8	2	1		2	2	2	2	2	2	5	3	13	3	14	5	
2	2		0.75	1	0	1	2	Left thigh	1	1	3	1		1	2	3	5	3	5	5	
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2	4		1.45	1	1	1	1		1	2	2	1		2	4	5		5		5	
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2	4		7.33	2	33	2	1		2	2	2	1		2	4	5		5		5	
2	4		1.4	2	7	1	1		1	2	2	1		2	4	1		1		3	3
2	2		4.58	2	10	2	2	Left calf	1	2	3	1		2	4	5		5		1	
2	2		7.68	2	23	2	1		1	2	2	1		2	4	3	3	3	3	1	
2	4		7.55	2	7	2	1		2	2	2	1		2	4	3	10	3	11	5	
2	6	Rhabdoid	12.83	2	23	1	1		1	2	2	1		2	4	1		3	52	5	

bonei nv	solel ast	scatt er	ne st	pigme nt	epicont our	latcirc um	cellsi ze	nucsi ze	nucleo lus	cellsha pe	mn gc	lndis sn	lnclins ite	lnsiteoth	local site	lnspecty pe	lnsize	maxlns ize
3	2	5	5	5	6	4	3	3	3	1	2	2						
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3	2	1	1	3	3	4	2	2	2	1	2	2						
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3	2	1	3	1	3	2	3	3	3	4	2	2						
3	2	3	3	5	5	2	3	3	3	1	2	2						
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3	2	2	2	5	5	2	3	3	3	1	2	2						
3	2	2	2	2	5	2	3	3	3	2	2	1	5	Right iliac	1	3	5.5x3.5x3	5.5
2	2	4	2	5	5	2	3	3	3	4	2	2						
1	2	3	3	2	5	2	3	3	3	4	2	2						
3	2	2	2	1	5	2	2	2	2	2	2	2						
3	2	2	3	4	5	2	3	3	3	1	2	2						
3	2	2	2	4	5	2	2	2	2	4	2	2						
3	2	1	1	1	5	4	3	3	3	1	2	2						
3	2	1	2	3	5	2	3	3	3	1	2	1	5	Lt iliac, obturator	2	3	3.5x3.5	3.5
2	2	2	2	5	5	1	3	3	3	4	2	2						
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3	2	4	2	5	5	2	3	3	3	2	2	2						
3	2	4	3	5	5	2	3	3	3	1	2	2						
3	2	4	3	5	5	2	3	3	3	1	2	2						
3	2	3	3	5	5	2	3	3	3	1	2	2						
2	2	2	1	5	5	2	3	3	3	4	2	1	1		2	3	4.4x2.9x2.1	4.4
2	2	2	2	1	5	2	3	3	3	1	2	2						

ther pln	therpl nidt	therpl ninv	therap icln	therec inv	therpln mar	tsta ge	nsta ge	ihcd ata	h m b	shu nd	mel ana	othihc	srldh	surg statu s	presl dh	preldh	pre ldh lev	postf uldh	postldh	postld hlev
						9	7	1	1				1	3	1	426	1	1	389.6	1
						6	7	1	1	1			2							
						8	5	1	1	1	1		1	3	1	482.2	2	1	437.7	1
						8	5	1	1	1			1	3	1	552.5	2	1	311	1
1	9	0	3	4	4	8	5	2					1	1	1	369.2	1	2		
						7	7	2					2							
						8	7	2					2							
						8	7	1	1			Cytoke ratin Neg	2							
						6	7	1	1		1	Viment in Pos	2							
						6	2	1	1		1	Cytoke ratin Neg	2							
						6	7	2					2							
						8	7	1	1	1			2							
						8	7	1	1	1	1		1	1	1	337	1	2		
						6	7	1	1	1		Cytoke ratin Neg	2							
						6	7	1	1	1			2							
						6	7	1	1				2							
						8	7	1	1				2							
						8	7	1	1	1		p63 Neg	2							
						8	7	2					2							
						8	5	1	2	1	1		2							
						6	7	1	1	1		p63 Neg	2							
						8	5	1	1	1		CD117 Pos	2							
1	17	1	3	1	4	5	2	1	1		1		2							
						8	5	1	1	1			2							
						8	7	1	1	1			2							
						1	7	1	1	1	1		2							
						6	5	1	1				2							
						6	7	1	1				2							
						3	7	1	1	1	1		2							
1	19	7	3	1	4	8	6	1		1			2							
						8	7	2					1	2	2			1	1451	2
						8	2	1	1		1		1	3	1	359	1	1	417	1
						1	7	1	1		1	CD117 Pos	2							
						6	7	1	1	1			2							
						7	7	1	1				2							
						5	7	1	1	1			2							
						8	7	2					2							
						8	7	1	1		1		2							
						4	7	2					2							
						8	5	1	1	1			2							
						8	7	1		1	1		2							
1	14	12	3	2	4	8	6	1	1	1			2							
						8	7	1	1	1			2							

fu	opdfirst	opdlast	fulast	survival	deathdate	clinstlitm	stmsite	clinstmpre	prelnfna	lnfnapres	adjtt	trtpres	recurrence	recbiopsy	recdate
1	Feb 2014	Sep 2014	Sep 2014	3		1	Rt calf, Rt thigh	1	3		1	3	2		
1	Feb 2014	Feb 2014	March 2014	1	March 2014	2			3		2		3		
1	March 2014	Oct 2014	May 2015	1	May 2015	1	Left thigh	1	3		2		1	1	Aug 2014
1	July 2014	July 2015	Oct 2015	1	Oct 2015	1	Lt foot, leg, thigh	1	2		1	1	2		
1	Aug 2014	Dec 2014	Dec 2014	3		1	Lt foot	1	2		2		2		
1	Feb 2015	Feb 2015	June 2015	1	June 2015	2			3		2		3		
1	March 2015	March 2016	April 2016	1	April 2016	2			3		1	1	2		
1	Oct 2014	April 2015	May 2015	1	May 2015	2			3		1	1	3		
2	July 2013	July 2013		3		2			3		2		3		
1	Aug 2013	Aug 2013	Sep 2014	1	Sep 2014	2			1	1	2		3		
1	Oct 2013	Feb 2014	Feb 2014	3		2			3		1	1	3		
1	Oct 2013	March 2016	Aug 2016	1	Aug 2016	2			2		2		2		
1	Nov 2015	June 2016	June 2016	3		2			2		2		1	2	June 2016
1	July 2013	July 2013	Aug 2017	2		2			3		2		3		
1	Jan 2015	Jan 2015	Dec 2015	1	Dec 2015	2			3		2		3		
2	Feb 2014	Feb 2014		3		2			3		2		3		
1	Oct 2013	Nov 2013	June 2014	1	June 2014	2			3		2		3		
1	Dec 2015	Dec 2015	Aug 2017	2		2			3		2		3		
1	Feb 2013	April 2016	April 2016	3		2			3		2		2		
1	Jan 2015	Feb 2015	Aug 2017	2		1	Left thigh	1	3		2		2		
2	July 2014	July 2014		3		1	Right foot	1	1	1	2		3		
1	Dec 2015	Feb 2016	Feb 2016	3		1	Lt thigh	2	3		2		3		
1	March 2013	June 2013	June 2013	3		2			1	2	2		2		
1	Dec 2014	May 2015	April 2016	1	April 2016	1	Rt foot, rt leg	1	3		2		2		
2	Nov 2015	Nov 2015		3		2			3		2		3		
1	July 2013	Dec 2013	Dec 2013	3		1	Left thigh	1	3		1	1	2		
1	Sep 2013	Oct 2013	Oct 2013	3		1	Rt foot	1	3		2		3		
1	Sep 2013	July 2014	Aug 2014	1	Aug 2014	2			3		1	1	2		
1	March 2013	Feb 2014	Feb 2014	3		2			2		1	2	1	2	Feb 2014
1	March 2013	April 2013	April 2013	3		2			1	1	2		3		
1	Oct 2013	July 2015	July 2015	3		2			1	1	2		2		
1	Oct 2015	June 2017	Aug 2017	2		2			1	1	1	1	2		
1	Feb 2013	Aug 2013	Oct 2015	1	Oct 2015	2			3		1	1	3		
1	Nov 2014	Jan 2017	Jan 2017	3		2			3		1	1	1	2	Jan 2017
1	March 2014	Sep 2014	May 2015	1	May 2015	2			3		2		3		
2	Aug 2015	Aug 2015		3		2			3		2		3		
2	Jan 2013	Jan 2013		3		2			3		2		3		
2	July 2013	July 2013		3		2			3		2		3		
2	July 2013	July 2013		3		2			3		2		3		
2	June 2014	June 2014		3		1	Left calf	1	3		2		3		
1	Aug 2014	Sep 2014	Oct 2015	1	Oct 2015	2			3		2		3		
1	Jan 2015	Feb 2016	Feb 2016	3		2			1	1	2		2		
1	May 2015	July 2015	Aug 2017	2		2			3		2		2		

metastases	mets pres	metsdate	distskinsc	skinsite	ln	lnsite	lung	liver	adrenal	skeletal	brain	othmets	metsbiopsy	biopsysite	mstage	cmstage	pmstage	braf	brafmut
1	2	Sep 2014	2		1	Peripancreatic	2	2	2	2	2		2		1	1		1	3
1	1		2		1	Mesenteric	2	1	2	2	2		2		1	3		1	2
1	2	Aug 2014	1	Chest wall	1	Right cervical	1	1	2	1	2	Spleen	1	Rt cervical LN	3	3	3	1	2
1	2	Feb 2015	2		2		2	1	2	2	2		2		1	3		1	2
2															1	4		1	2
2															1	4		1	2
1	1		2		2		1	1	1	1	2		2		1	3		1	2
1	1		2		1	Lt inguinal, Lt int iliac	2	1	2	1	2		2		1	3		1	2
1	1		2		2		2	1	2	2	2		2		1	3		1	2
2															1	4		1	2
1	1		2		2		1	1	2	1	2		2		1	3		1	2
1	2	March 2016	2		1	Rt int iliac	2	1	2	2	2		2		1	3		1	2
1	2	June 2016	2		1	Right axillary	1	1	2	2	2		2		1	3		1	2
1	1		2		2		1	2	2	2	2		2		1	2		1	2
2															1	4		1	2
2															1	4		1	2
1	1		2		2		2	1	2	2	2		2		1	3		1	2
2															1	4		1	2
2															1	4		1	3
1	2	Feb 2015	2		2		1	2	2	2	2		2		1	2		1	2
1	1		2		2		2	1	2	2	2		2		1	3		1	2
1	2	Feb 2016	1	Chest wall	2		2	2	2	2	2		2		1	1		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
1	2	Aug 2013	2		2		2	2	2	1	2		1	Rt iliac bone	3	3	3	1	3
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
1	2	July 2015	2		2		1	1	2	1	2	Pancreas, Kidney	2		1	3		1	2
1	2	June 2016	2		2		2	1	2	2	2		2		1	3		1	2
1	2	Aug 2013	2		2		2	1	2	2	2		2		1	3		1	2
1	1		2		2		1	1	2	1	2		2		1	3		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2
2															1	4		1	2

serno	biopno	hospno	age	gender	residence	clinsite	spectype	size	maxtumour	insitu	histotype	regres	regpres	permarg	pmarni	deepmarg
45	33429/15	296635G	58	2	West Bengal	Right heel	1	0.6x0.6	0.6	2	2	1		5		5
46	13602/13	460282F	40	1	West Bengal	Left foot	6	NA		2	2	1		5		5
47	16778/14	084643A	75	1	Tamilnadu	Right heel	6	NA		2	2	1		5		5
48	20297/13	488510F	47	1	Tamilnadu	Anal canal	2	1.5x1.5	1.5	3						
49	15611/13	471123F	75	2	West Bengal	Right leg	1	0.4x0.4	0.4	3						
50	2635/14	121850F	59	2	Tamilnadu	Left heel	6	NA		2	1	2		5		5
1	5719/14	789929F	45	2	Tamilnadu	Right leg	5	8.9x4.9x4.5	8.9	2	2	1		5		5
2	33099/14	821956F	47	2	Tamilnadu	Left heel	1	5x4	5	2	2	1		3	1	5
3	32863/14	003937G	28	1	West Bengal	Left foot	3	1.7x1.3x1	1.7	2	2	1		3	18	5
4	19338/15	949063F	48	2	Tamilnadu	Anal canal	5	4.5x2.5x2	4.5	3						
5	45254/13	981366D	55	1	Bihar	Anal canal	4	NA		2	2	1		1		5
6	46066/15	345125G	57	1	West Bengal	Anal canal	4	4x3.5x2	4	2	1	1		3	20	5
7	28598/14	000758G	51	2	West Bengal	Right foot	4	5.5x5x3	5.5	2	2	2	2	1		5
8	14315/13	432991F	33	2	Assam	Right foot	3	4.8x4.3x0.8	4.8	2	2	1		3	9	5
9	28557/13	634589F	75	1	West Bengal	Left little toe	4	1.9x1.5x0.8	1.9	2	2	1		3	20	5
10	34294/13	670148F	40	1	West Bengal	Right heel	4	4x3x1.2	4	2	2	2	1	1		5
11	34945/13	666509F	43	1	West Bengal	Right foot	4	0.5x0.4x0.3	0.5	2	2	2	1	3	30	5
12	13472/13	223233F	46	1	West Bengal	Anal canal	4	6x4.5x2.5	6	2	2	1		1		5
13	37706/13	706227F	65	1	Jharkand	Left great toe	1	0.6x0.5x0.2	0.6	2	2	1		1		5
14	41660/15	322980G	62	1	Chattisgarh	Right foot	4	3.6x3.6x1.1	3.6	2	2	2	2	1		5
15	48934/14	100258G	45	2	West Bengal	Anal canal	5	2x1.5x0.6	2	2	2	1		5		5
16	8834/13	397591F	36	1	West Bengal	Left index finger	4	7x6x5	7	2	2	1		1		5
17	36459/15	296635G	58	2	West Bengal	Right heel	3	3x2.5x1	3	2	2	1		3	13	5
18	15066/13	460282F	40	1	West Bengal	Left foot	3	3x0.5x0.4	3	2	2	1		3	13	5
19	17609/14	084643A	75	1	Tamilnadu	Right heel	3	2.2x2x0.3	2.2	2	2	1		3	2	5
20	16288/13	471123F	75	2	West Bengal	Right leg	3	10x6x1.5	10	2	1	1		1		5
21	6048/14	121850F	59	2	Tamilnadu	Left heel	3	1x1x0.8	1	1						
22	19622/15	228577G	86	1	Tamilnadu	Right little finger	6	NA		2	2	1		5		5
1	46177/14	100258G	45	2	West Bengal	Anal canal	2	0.2x0.2	0.2	3						
2	1013/14	460282F	41	1	West Bengal	Left foot	5	0.1x0.1	0.1	1						

invasive	histotype1	subtype	breslow	ulcer	mitoses	lvi	stltrans	stmpres	pni	growth	til	regression	rgrp res	clark	clarkno	inspmarg	inspmni	invpmarg	invpmni	invdmarg	invdmni
2	2		3.23	2	7	2	1		1	2	2	1		2	4	5		5		5	
2	4		10.53	2	4	1	1		1	2	2	1		2	4	5		5		5	
2	2		3	2	3	2	1		2	2	2	1		2	4	5		5		5	
2	4		1.3	2	1	1	1		1	2	2	1		2	4	5		5		5	
2	4		1.08	2	1	1	1		2	1	2	1		2	5	5		5		5	
2	4		3.28	2	4	1	1		1	2	2	1		2	4	5		5		5	
2	4		45	1	12	2	2	Right thigh	1	2	1	1		2	4	5		5		1	
2	2		4.15	2	29	1	1		1	2	2	1		2	4	3	1	3	2	1	
2	2		7.88	2	18	1	1		1	2	3	1		2	5	3	18	3	18	3	3
2	4		20	2	48	2	1		2	2	3	1		2	5	5		5		5	
1				2		1	1		4			1				5		5		5	
2	4		25	2	52	2	1		1	2	2	1		2	5	3	20	3	20	2	
2	2		55	2	12	2	2	Right foot	2	2	2	2	2	2	5	1		3	14	4	
2	6	Spitzoid	6.35	2	27	2	1		1	2	3	1		2	4	3	9	3	11	3	7
2	2		7.13	2	3	1	1		2	2	3	1		2	5	3	20	3	20	5	
2	2		4.95	2	20	1	2	Right foot	1	2	3	2	1	2	4	1		3	180	5	
2	2		2.3	2	0	2	1		1	2	2	2	1	2	4	3	30	3	30	4	
2	4		25	2	8	2	1		2	2	2	1		2	5	1		3	20	3	2
2	2		3.2	2	7	1	1		1	2	2	1		2	4	1		1		3	1
2	2		9.1	2	14	2	1		2	2	2	2	2	2	5	1		3	17	5	
2	4		12.6	2	8	2	1		2	2	2	1		2	5	5		5		1	
2	2		50	2	59	2	1		1	2	2	1		2	4	1		3	70	5	
2	2		8.83	2	23	2	1		2	2	2	1		2	5	3	13	3	14	3	6
2	4		0.78	1	0	1	1		1	1	2	1		1	2	3	13	3	13	4	
2	2		3.7	2	17	2	1		2	2	2	1		2	4	3	2	3	2	3	3
2	4		15	2	9	2	2	Right leg	2	2	2	1		2	5	1		3	10	2	
2	6	Balloon cell	6.28	2	13	2	2	Left heel	1	2	2	1		2	4	5		3	4	3	5
2	2		0.78	2	16	1	1		1	1	2	1		1	2	5		5		5	
2	4		0.3	2	3	1	1		1	1	2	1		1	3	5		5		5	
2	4		0.65	2	0	1	1		1	1	2	1		1	3	5		3	6	3	2

bonei nv	solel ast	scatt er	ne st	pigm ent	epicont our	latcirc um	cellsi ze	nucsi ze	nucleo lus	cellsha pe	mn gc	lndis sn	lnclins ite	lnsiteoth	local site	lnspecty pe	lnsize	maxlns ize
3	2	3	2	5	5	2	3	3	3	2	2	2						
3	2	2	3	3	5	2	3	3	3	2	2	2						
3	2	2	2	5	5	2	2	2	2	1	2	2						
3	2	5	5	5	6	4	3	3	3	1	2	2						
3	2	5	5	5	6	4	3	3	3	2	2	2						
3	2	1	1	1	5	4	3	3	3	1	2	1	5	Left iliac	2	3	4x3x2	4
3	2	2	1	5	4	2	3	3	3	1	2	2						
3	2	3	3	1	3	2	3	3	3	2	2	2						
3	2	2	2	1	5	2	3	3	3	4	2	1	5	Left iliac	2	3	10x6x6	10
3	2	5	5	1	6	4	2	2	2	2	2	2						
3	2	3	1	5	5	4					2	1	3	Mesocolic	3	3	0.5x0.5	0.5
3	2	2	2	3	4	2	3	3	3	3	2	1	3	Mesocolic	3	3	1.2x1.2	1.2
2	2	2	2	1	5	2	3	3	3	2	2	1	2		1	3	4.5x4.5 Matted	4.5
3	2	2	2	5	5	2	3	3	3	4	1	2						
2	2	3	2	1	5	2	2	2	2	3	1	2						
2	2	3	3	4	5	2	3	3	3	1	2	2						
2	2	2	2	5	5	2	2	2	2	4	2	2						
3	2	2	1	1	5	2	3	3	3	1	2	1	3	Perirectal	3	3	3x2x1.5	3
3	2	2	1	5	5	1	3	3	3	2	2	2						
2	2	2	2	4	5	2	3	3	3	4	1	1	2		1	3	1x1	1
3	2	2	2	3	2	2	3	3	3	1	2	2						
2	2	2	2	1	5	2	3	3	3	4	2	1	1		2	3	6.5x6.5	6.5
3	2	3	2	5	5	2	3	3	3	4	2	2						
3	2	2	3	3	5	2	3	3	3	2	2	1	2		2	3	2.5x2.5	2.5
3	2	2	2	5	5	2	3	3	3	1	2	2						
3	2	2	1	5	5	2	3	3	3	4	2	1	2		1	3	1x1	1
3	2	1	1	1	5	4	3	3	3	1	2	2						
3	2	2	2	1	5	2	3	3	3	1	2	2						
3	2	5	5	3	6	4	2	2	2	1	2	2						
3	2	1	1	2	3	4	3	3	3	2	2	2						

ther pln	therpl nidt	therpl ninv	therap icln	therec inv	therpln mar	tsta ge	nsta ge	ihcd ata	h m b	shu nd	mel ana	othihc	srldh	surg statu s	presl dh	preldh	pre ldh lev	postf uldh	postldh	postld hlev
						6	5	1	1				2							
						8	2	1	1	1	1		1	1	1	449	1	2		
						6	7	2					2							
						4	7	1	1	1			2							
						4	5	1	1		1		2							
1	16	0	3	4	4	6	8	1	2	1	2		2							
						7	5	2												
						8	7	1	1											
1	20	5	3	2	4	8	6	2												
						8	7	1	1											
1	16	0	3	4	4	11	8	2												
1	16	8	1	2	4	8	6	2												
1	2	2	3	2	4	8	6	1	1											
						8	7	2												
						8	7	2												
						8	5	2												
						6	7	2												
1	10	5	3	1	4	8	6	1	1											
						6	7	1	1	1	1									
1	16	9	3	2	4	8	6	2												
						8	7	1	1											
1	5	5	3	1	4	8	6	2												
						8	7	2												
1	15	6	3	2	4	1	6	2												
						6	7	2												
1	2	2	3	1	4	8	6	1				1								
						8	5	2												
						2	7	2												
						2	7	1	1											
						2	7	1	1			1								

fu	opdfirst	opdlast	fulast	survival	deathdate	clinstlitm	stmsite	clinstmpre	prelnfna	lnfnapres	adjtrt	trtpres	recurrence	recbiopsy	recdate
1	Aug 2015	Sep 2015	Sep 2016	1	Sep 2016	1	Right heel	1	3		2		2		
1	April 2013	March 2016	Aug 2016	1	Aug 2016	1	Lt shin, popliteal	2	1	1	1	2	1	1	Jan 2014
1	May 2014	Aug 2017	Aug 2017	2		2			3		2		2		
2	May 2013	May 2013		3		2			3		2		3		
1	May 2013	July 2013	May 2015	1	May 2015	1	Right leg	1	2		2		2		
1	Jan 2014	April 2014	Aug 2017	2		2			2		2		2		

metastases	metspres	metsdate	distskinsc	skinsite	ln	lnsite	lung	liver	adrenal	skeletal	brain	othmets	metsbiopsy	biopsysite	mstage	cmstage	pmstage	braf	brafmut
2															1	4		1	2
1	2	March 2016	2		2		2	2	2	2	2	Colon-Hep flexure	2		1	3		1	2
1	2	Aug 2017	2		1	Paraca val	1	1	2	2	2		2		1	3		1	2
2															1	4		1	3
2															1	4		1	3
2															1	4		1	2